

09/865363

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No. 6,740,522
Issue date May 25, 2004
Inventors Dirk M. Anderson
For ANTIBODIES AGAINST LIGAND FOR RECEPTOR ACTIVATOR OF NF-KB

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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PATENT EXTENSION
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Dear Sir:

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Immunex Corporation, a Washington Corporation, and a wholly-owned subsidiary of Applicant Amgen Inc., a Delaware Corporation, represents that it is the assignee of the entire interest in and to Letters Patent of the United States No. 6,740,522 granted to Dirk M. Anderson on May 25, 2004. The assignment (for parent application US Application No. 08/995,659 to which U.S. Patent No. 6,740,522 claims priority), from the inventor to Immunex Corporation was recorded on March 27, 2000, Reel 010732, Frame 0297.

Notice Regarding Multiple Applications

Multiple applications for term extension are being filed based on the regulatory review period for PROLIA™ (denosumab). The patents involved are:

6,740,522
7,097,834
7,411,050
7,449,185
7,527,790.

Applicant is aware that the term of only one patent may be extended for each regulatory review period. 35 U.S.C. § 156(c)(4). An election of only one patent will be made in accordance with 37 C.F.R. § 1.785(b) upon receipt of a notice of final determination in these applications from the U.S. Patent and Trademark Office. Applicant requests concurrent processing of these applications by both the U.S. Patent and Trademark Office and the Food and Drug Administration.

Information Required Under 37 C.F.R. § 1.740

Applicant hereby submits this application for an extension of patent term under 35 U.S.C. § 156 by providing the following information as required by § 1.740 of Title 37 of the code of Federal Regulations (37 C.F.R. § 1.740).

1. The approved product is PROLIA™, a trademark owned by Amgen Inc., for denosumab, a fully human monoclonal antibody which binds to human RANKL.
2. The approved product was subject to regulatory reviews under Section 505 of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 355.
3. The approved product PROLIA™ (denosumab) received permission for commercial marketing or use under § 505 of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 355, on June 1, 2010.
4. The active ingredient in the approved product PROLIA™ is denosumab which has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act. No other active ingredients are contained in this product.
5. This application for extension of patent term under 35 U.S.C. § 156 is being submitted within the sixty (60) day period permitted for submission pursuant to § 1.720(f). The last day for submitting an application for extension is July 30, 2010.
6. The complete identification of the patent for which an extension is being sought is as follows:

Inventors	Dirk M. Anderson
Patent No.	6,740,522
Issue date	May 25, 2004
Expiration date	December 22, 2017

7. A copy of the patent for which an extension is being sought is attached hereto as Attachment "A".

8. A receipt for maintenance fee payment for this patent is attached hereto as Attachment "B". A certificate of correction is attached hereto as Attachment "C". No terminal disclaimer or reexamination certificate has been issued with respect to U.S. Patent No. 6,740,522.

9. The patent claims the approved product PROLIA™ (denosumab) in at least claims 1-2, 6-9 and 11-14.

In particular, Claims 1, 7 and 14 read on the approved product as follows:

1. A purified antibody that binds with higher affinity to a human RANKL polypeptide as shown in SEQ ID NO. 13 than to a murine RANKL polypeptide as shown in SEQ ID NO. 11.

7. A purified antibody that binds with higher affinity to a human RANKL polypeptide than to a murine RANKL polypeptide according to SEQ ID NO. 11, wherein said human RANKL polypeptide is selected from the group consisting of: a) a RANKL polypeptide comprising amino acids 69-313 of SEQ ID NO. 13; b) a RANKL polypeptide comprising amino acids 1-162 of SEQ ID NO. 13; c) a RANKL polypeptide comprising amino acids 162-313 of SEQ ID NO. 13; d) a RANKL polypeptide comprising amino acids 138-317 of SEQ ID NO. 13; and e) a RANKL polypeptide comprising amino acids x to y of SEQ ID NO. 13, wherein x is an amino terminal amino acid between 69 and 162 of SEQ ID NO. 13, and y is a carboxy terminal amino acid between 313 and 317 of SEQ ID NO. 13.

14. A purified antibody that binds to a human RANKL polypeptide as shown in SEQ ID NO. 13, but that does not bind to a murine RANKL polypeptide as shown in SEQ ID NO. 11.

A copy of the U.S. package insert for PROLIA™ is attached hereto as Attachment "D."

10. The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

- (i) the effective date of the investigational new drug (IND) application was June 21, 2001;
- (ii) the IND number was BB IND 9837;
- (iii) the date on which a biologic license application (BLA) was initially submitted was December 19, 2008;
- (iv) the BLA number was BL 125320; and
- (v) the date on which the BLA was approved was June 1, 2010.

11. The following is a brief description of the significant activities undertaken by the marketing applicant (Amgen Inc.) during the applicable regulatory review period with respect to PROLIA™ (denosumab) and the significant dates applicable to such activities:

- (i) the effective date of the IND filing was June 21, 2001;
- (ii) the clinical trials were performed in subjects having osteoporosis;
- (iii) the BLA application was submitted December 19, 2008; and
- (iv) the BLA was approved on June 1, 2010.

A chronology of the activities undertaken by Amgen Inc. with respect to PROLIA™ from the effective date of the IND to the approval of the BLA is set forth in Attachment "E".

12(A). It is the opinion of the Applicant that U.S. Patent No. 6,740,522 claims the approved product that has undergone a regulatory review which would be considered in determining any extension for patent under 35 U.S.C. § 156 for the following reasons:

- (i) U.S. Patent No. 6,740,522 claims the approved product (35 U.S.C. § 156(a));
- (ii) The term of U.S. Patent No. 6,740,522 has not expired before submission of this application for an extension (35 U.S.C. § 156(a)(1));
- (iii) The term of U.S. Patent No. 6,740,522 has never been previously extended (35 U.S.C. § 156(a)(2));
- (iv) The application for extension is submitted by Amgen, the parent company of the owner of record of U.S. Patent No. 6,740,522 in accordance with the requirements of 35 U.S.C. § 156(d), 37 C.F.R. § 1.730, and 37 C.F.R. § 1.740 (35 U.S.C. § 156(a)(3));
- (v) The product PROLIA™ (denosumab) has been subject to a regulatory review period before its commercial marketing or use (35 U.S.C. § 156(a)(4));
- (vi) The product PROLIA™ (denosumab) has received permission for commercial marketing or use, and the permission for the commercial marketing or use of the product after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of the Federal Food, Drug and Cosmetic Act, under which the regulatory review period occurred (35 U.S.C. § 156(a)(5)(A));
- (vii) No other patent has been extended for the same regulatory review period for the product PROLIA™ (denosumab) (35 U.S.C. § 156(c)(4)); and
- (viii) Amgen, the parent company of the owner of record of U.S. Patent No. 6,740,522 has hereby submitted an application to the Commissioner to obtain an extension of the term of the patent within the sixty (60) day period beginning on the date the product received permission for commercial marketing or use (35 U.S.C. § 156(d)(1)).

12(B). The length of extension of the patent term of U.S. Patent No. 6,740,522 claimed by Applicants is 1365 days. The length of extension was determined by the following:

- (i) The U.S. Patent No. 6,740,522 issued May 25, 2004, which was after the date of enactment of 35 U.S.C. § 156. The commercial marketing or use of the product, PROLIA™ (denosumab), was approved after the date of enactment of 35 U.S.C. § 156.
- (ii) The regulatory review period under 35 U.S.C. § 156(g)(1)(B) was from June 21, 2001, until June 1, 2010, which was 3268 days.
- (iii) The period of review under 35 U.S.C. § 156(g)(1)(B)(i) began on the date an exemption under § 505(i) became effective on June 21, 2001 and ended on the date an application was initially submitted for PROLIA™ (denosumab) under § 505 which was December 19, 2008, a total of 2739 days.
- (iv) The regulatory review period under 35 U.S.C. § 156(g)(1)(B)(ii) began on the date the application was initially submitted for PROLIA™ (denosumab), under § 505(b), which was December 19, 2008 and ended on the date such application was approved under such section, which was June 1, 2010, a total of 530 days.
- (v) The issuance of U.S. Patent No. 6,740,522 occurred on May 25, 2004, which was 1070 days after the effective date of the IND application (June 21, 2001).
- (vi) In compliance with § 1.775(d)(1)(i), the number of days in the period set forth in item (v) of this paragraph 12(B), i.e., 1070 days, is subtracted from the period determined under 35 U.S.C. § 156(g)(1)(B)(i), which is set forth in item (iii) of this paragraph 12(B), i.e., 2739 days, to provide an adjusted regulatory period under 35 U.S.C. § 156(g)(1)(B)(i) of 1669 days.
- (vii) Under 35 U.S.C. § 156(c)(2), the period of extension includes only one-half of the period determined under 35 U.S.C. § 156(g)(1)(B)(i), which is set forth in item (vi) of this paragraph 12(B), which is 835 days, together with the number of days required for approval set forth in item (iv) of this paragraph 12(B), i.e., 530 days, for an extension of 1365 days.
- (viii) In compliance with 35 U.S.C. § 156(c)(3), the period remaining in the term of U.S. Patent No. 6,740,522 after BLA approval of PROLIA™ (denosumab) is from June 1, 2010 to December 22, 2017, or 2762 days, which when added to the period of extension under item (vii) of this paragraph 12(B), i.e., 1365 days, is a total of 4127 days, or 11 years and 111 days, which is not in excess of fourteen

(14) years provided in 35 U.S.C. § 156(c)(3). Therefore, the period of extension claimed by Applicant is 1365 days which would result in an expiration date of September 21, 2021.

13. Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought in this application.

14. The prescribed fee for receiving and acting upon the application for extension of \$1,120.00 is enclosed with this application. Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 06-0916 for any such fees. Should a refund of fee paid be necessary, the Commissioner is hereby authorized to credit any such amount to Deposit Account No. 06-0916.

15. Inquiries and correspondence relating to this application for patent term extension are to be directed to the correspondence address associated with Customer No. 22852:

Charles E. Van Horn
Finnegan, Henderson, Farabow,
Garrett & Dunner, LLP
901 New York Avenue, N.W.
Washington, D.C. 20001
Telephone No. 202-408-4072

16. Two additional copies of the application papers for extension of the patent term of U.S. Patent No. 6,740,522 are enclosed with the application.

17. The undersigned is a registered practitioner of the United States Patent and Trademark Office and is authorized by the Applicant to act on behalf of the Applicant.

Respectfully submitted,

Date: 07/27/10

Charles E. Van Horn
Charles E. Van Horn
Reg. No. 40,266

Attachment A: Copy of USP 6,740,522
Attachment B: Maintenance Fee Statement for USP 6,740,522
Attachment C: Certificate of Correction for USP 6,740,522
Attachment D: Package Insert for PROLIA™
Attachment E: Brief Description of Regulatory Activities

CERTIFICATION

The undersigned hereby certifies that this application for extension of patent term under 35 U.S.C. § 156, including its attachments and supporting papers, is being submitted with two additional copies of originals.

Date 07/27/10

Charles E. Van Horn

Charles E. Van Horn
Reg. No. 40,266

ATTACHMENT A

In re U.S. Patent No. 6,740,522

Issued: May 25, 2004

To: Dirk M. Anderson

Assignee: Immunex Corporation

**For: ANTIBODIES AGAINST LIGAND FOR RECEPTOR
ACTIVATOR OF NF-KB**

Application for Patent Term Extension

Customer No. 22852

(12) **United States Patent**
Anderson

(10) **Patent No.:** **US 6,740,522 B2**
(45) **Date of Patent:** **May 25, 2004**

(54) **ANTIBODIES AGAINST LIGAND FOR
RECEPTOR ACTIVATOR OF NF-KB**

(75) **Inventor:** **Dirk M. Anderson, Seattle, WA (US)**

(73) **Assignee:** **Immunex Corporation, Seattle, WA
(US)**

(*) **Notice:** Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** **09/865,363**

(22) **Filed:** **May 25, 2001**

(65) **Prior Publication Data**

US 2002/0086826 A1 Jul. 4, 2002

Related U.S. Application Data

(62) Division of application No. 09/577,780, filed on May 24,
2000, now Pat. No. 6,419,929, which is a division of
application No. 08/995,659, filed on Dec. 22, 1997, now Pat.
No. 6,242,213.

(60) Provisional application No. 60/064,671, filed on Oct. 14,
1997, provisional application No. 60/077,181, filed on Mar.
7, 1997, and provisional application No. 60/059,978, filed
on Dec. 23, 1996.

(51) **Int. Cl.⁷** **C12N 5/20**

(52) **U.S. Cl.** **435/335; 435/331; 435/326;
435/346; 530/351; 530/388.23; 514/12**

(58) **Field of Search** **530/351, 388.22,
530/388.23, 388.1**

(56) **References Cited**

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Primary Examiner—Lorraine Spector

Assistant Examiner—Eileen B. O'Hara

(74) **Attorney, Agent, or Firm**—Diana K. Sheiness

(57) **ABSTRACT**

Provided herein are antibodies that bind human RANKL
polypeptides.

22 Claims, 2 Drawing Sheets

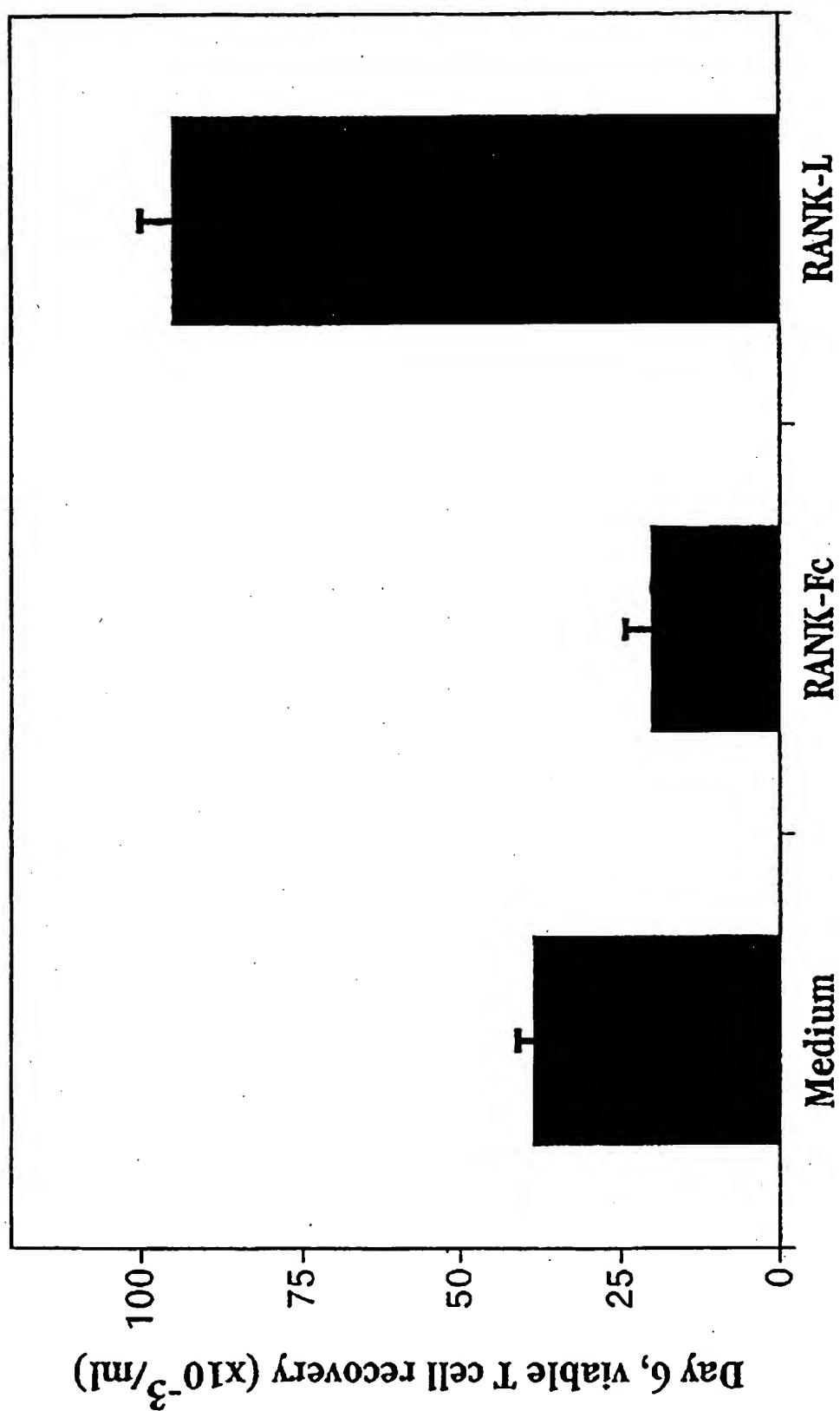


FIGURE 1

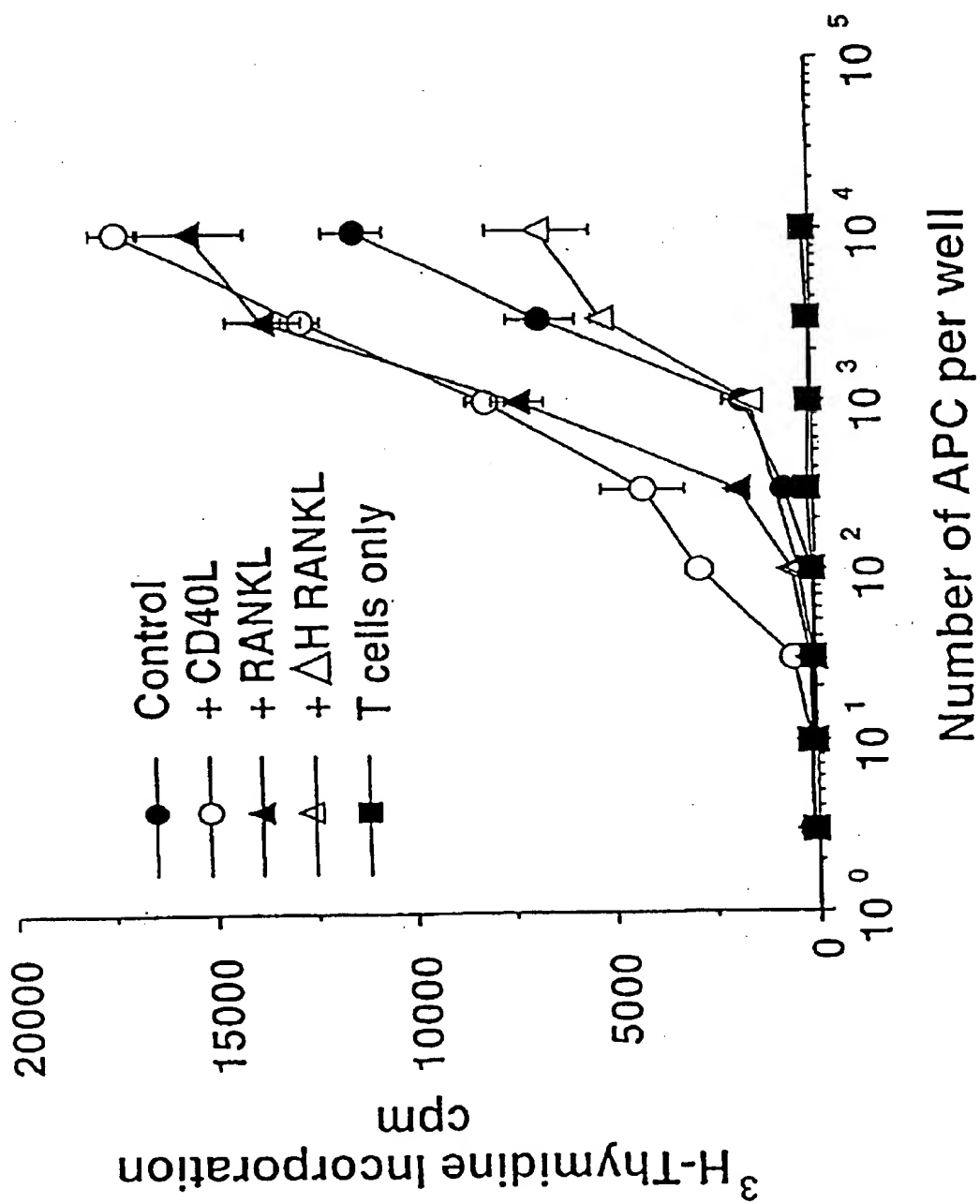


FIGURE 2

ANTIBODIES AGAINST LIGAND FOR RECEPTOR ACTIVATOR OF NF- κ B

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This application is a divisional of U.S. Ser. No. 09/577,780, filed May 24, 2000, now U.S. Pat. No. 6,419,929, which is a divisional of U.S. Ser. No. 08/995,659, filed Dec. 22, 1997, now U.S. Pat. No. 6,242,213, which claims the benefit of U.S. Ser. No. 60/064,671, filed Oct. 14, 1997, U.S. Ser. No. 60/077,181, filed Mar. 7, 1997 (filed originally as U.S. Ser. No. 08/813,509), and U.S. Ser. No. 60/059,978, filed Dec. 23, 1996 (filed originally as U.S. Ser. No. 08/772,330).

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the field of cytokines, and more specifically to cytokine receptor/ligand pairs having immunoregulatory activity.

BACKGROUND OF THE INVENTION

Efficient functioning of the immune system requires a fine balance between cell proliferation and differentiation and cell death, to ensure that the immune system is capable of reacting to foreign, but not self antigens. Integral to the process of regulating the immune and inflammatory response are various members of the Tumor Necrosis Factor (TNF) Receptor/Nerve Growth Factor Receptor superfamily (Smith et al., *Science* 248:1019; 1990). This family of receptors includes two different TNF receptors (Type I and Type II; Smith et al., supra; and Schall et al., *Cell* 61:361, 1990), nerve growth factor receptor (Johnson et al., *Cell* 47:545, 1986), B cell antigen CD40 (Stamenkovic et al., *EMBO J.* 8:1403, 1989), CD27 (Camerini et al., *J. Immunol.* 147:3165, 1991), CD30 (Durkop et al., *Cell* 68:421, 1992), T cell antigen OX40 (Mallett et al., *EMBO J.* 9:1063, 1990), human Fas antigen (Itoh et al., *Cell* 66:233, 1991), murine 4-1BB receptor (Kwon et al., *Proc. Natl. Acad. Sci. USA* 86:1963, 1989) and a receptor referred to as Apoptosis-Inducing Receptor (AIR; U.S. Ser. No. 08/720,864, filed Oct. 4, 1996).

CD40 is a receptor present on B lymphocytes, epithelial cells and some carcinoma cell lines that interacts with a ligand found on activated T cells, CD40L (U.S. Ser. No. 08/249,189, filed May 24, 1994). The interaction of this ligand/receptor pair is essential for both the cellular and humoral immune response. Signal transduction via CD40 is mediated through the association of the cytoplasmic domain of this molecule with members of the TNF receptor-associated factors (TRAFs; Baker and Reddy, *Oncogene* 12:1, 1996). It has recently been found that mice that are defective in TRAF3 expression due to a targeted disruption in the gene encoding TRAF3 appear normal at birth but develop progressive hypoglycemia and depletion of peripheral white cells, and die by about ten days of age (Xu et al., *Immunity* 5:407, 1996). The immune responses of chimeric mice reconstituted with TRAF3^{-/-} fetal liver cells resemble those of CD40-deficient mice, although TRAF3^{-/-} B cells appear to be functionally normal.

The critical role of TRAF3 in signal transduction may be in its interaction with one of the other members of the TNF receptor superfamily, for example, CD30 or CD27, which are present on T cells. Alternatively, there may be other, as yet unidentified members of this family of receptors that interact with TRAF3 and play an important role in postnatal

development as well as in the development of a competent immune system. Identifying additional members of the TNF receptor superfamily would provide an additional means of regulating the immune and inflammatory response, as well as potentially providing further insight into post-natal development in mammals.

SUMMARY OF THE INVENTION

The present invention provides a counterstructure, or ligand, for a novel receptor referred to as RANK (for receptor activator of NF- κ B), that is a member of the TNF superfamily. The ligand, which is referred to as RANKL, is a Type 2 transmembrane protein with an intracellular domain of less than about 50 amino acids, a transmembrane domain and an extracellular domain of from about 240 to 250 amino acids. Similar to other members of the TNF family to which it belongs, RANKL has a 'spacer' region between the transmembrane domain and the receptor binding domain that is not necessary for receptor binding. Accordingly, soluble forms of RANKL can comprise the entire extracellular domain or fragments thereof that include the receptor binding region.

RANK is a Type I transmembrane protein having 616 amino acid residues that is a member of the TNFR superfamily, and interacts with TRAF3. Triggering of RANK by over-expression, co-expression of RANK and membrane bound RANKL, or by soluble RANKL or agonistic antibodies to RANK, results in the upregulation of the transcription factor NF- κ B, a ubiquitous transcription factor that is most extensively utilized in cells of the immune system.

These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 demonstrates the influence of RANK.Fc and hRANKL on activated T cell growth. Human peripheral blood T cells were cultured as described in Example 12; viable T cell recovery was determined by triplicate trypan blue counts.

FIG. 2 demonstrates that RANKL enhances DC allostimulatory capacity. Allogeneic T cells were incubated with varying numbers of irradiated DC cultured as described in Example 13. The cultures were pulsed with [³H]-thymidine and the cells harvested onto glass fiber sheets for counting. Values represent the mean \pm standard deviation (SD) of triplicate cultures.

DETAILED DESCRIPTION OF THE INVENTION

A novel partial cDNA insert with a predicted open reading frame having some similarity to CD40 was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendritic cells (DC). The insert was used to hybridize to colony blots generated from a DC cDNA library containing full-length cDNAs. Several colony hybridizations were performed, and two clones (SEQ ID NOs: 1 and 3) were isolated. SEQ ID NO: 5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOs: 1 and 3.

RANK is a member of the TNF receptor superfamily; it most closely resembles CD40 in the extracellular region. Similar to CD40, RANK associates with TRAF2 and

TRAF3 (as determined by co-immunoprecipitation assays substantially as described by Rothe et al., *Cell* 83:1243, 1995). TRAFs are critically important in the regulation of the immune and inflammatory response. Through their association with various members of the TNF receptor superfamily, a signal is transduced to a cell. That signal results in the proliferation, differentiation or apoptosis of the cell, depending on which receptor(s) is/are triggered and which TRAF(s) associate with the receptor(s); different signals can be transduced to a cell via coordination of various signaling events. Thus, a signal transduced through one member of this family may be proliferative, differentiative or apoptotic, depending on other signals being transduced to the cell, and/or the state of differentiation of the cell. Such exquisite regulation of this proliferative/apoptotic pathway is necessary to develop and maintain protection against pathogens; imbalances can result in autoimmune disease.

RANK is expressed on epithelial cells, some B cell lines, and on activated T cells. However, its expression on activated T cells is late, about four days after activation. This time course of expression coincides with the expression of Fas, a known agent of apoptosis. RANK may act as an anti-apoptotic signal, rescuing cells that express RANK from apoptosis as CD40 is known to do. Alternatively, RANK may confirm an apoptotic signal under the appropriate circumstances, again similar to CD40. RANK and its ligand are likely to play an integral role in regulation of the immune and inflammatory response.

Moreover, the post-natal lethality of mice having a targeted disruption of the TRAF3 gene demonstrates the importance of this molecule not only in the immune response but in development. The isolation of RANK, as a protein that associates with TRAF3, and its ligand, RANKL, will allow further definition of this signaling pathway, and development of diagnostic and therapeutic modalities for use in the area of autoimmune and/or inflammatory disease. DNAs, Proteins and Analogs

The present invention provides isolated RANKL polypeptides and analogs (or muteins) thereof having an activity exhibited by the native molecule (i.e., RANKL muteins that bind specifically to a RANK expressed on cells or immobilized on a surface or to RANKL-specific antibodies; soluble forms thereof that inhibit RANK ligand-induced signaling through RANK). Such proteins are substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. Derivatives of RANKL within the scope of the invention also include various structural forms of the primary proteins which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a RANKL protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction. The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C-termini.

Derivatives of RANKL may also be obtained by the action of cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and lysine residues. The inventive proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated, carbonyldiimidazole-activated or

tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, the proteins may be used to selectively bind (for purposes of assay or purification) antibodies raised against the proteins or against other proteins which are similar to RANKL, as well as other proteins that bind RANKL or homologs thereof.

Soluble forms of RANKL are also within the scope of the invention. The nucleotide and predicted amino acid sequence of the RANKL is shown in SEQ ID Nos: 11 and 13 (murine and human, respectively). Computer analysis indicated that the RANKL is a Type 2 transmembrane protein; murine RANKL contains a predicted 48 amino acid intracellular domain, 21 amino acid transmembrane domain and 247 amino acid extracellular domain, and human RANKL contains a predicted 47 amino acid intracellular domain, 21 amino acid transmembrane domain and 249 amino acid extracellular domain.

Soluble RANKL comprises a signal peptide and the extracellular domain or a fragment thereof. An exemplary signal peptide is that shown in SEQ ID NO: 9; other signal (or leader) peptides are well-known in the art, and include that of murine Interleukin-7 or human growth hormone. RANKL is similar to other members of the TNF family in having a region of amino acids between the transmembrane domain and the receptor binding region that does not appear to be required for biological activity; this is referred to as a 'spacer' region. Amino acid sequence alignment indicates that the receptor binding region is from about amino acid 162 of human RANKL to about amino acid 317 (corresponding to amino acid 139 through 294 of murine RANKL, SEQ ID NO: 11), beginning with an Ala residue that is conserved among many members of the family (amino acid 162 of SEQ ID NO: 13).

Moreover, fragments of the extracellular domain will also provide soluble forms of RANKL. Those skilled in the art will recognize that the actual receptor binding region may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of a soluble RANKL is expected to be within about five amino acids on either side of the conserved Ala residue. Alternatively, all or a portion of the spacer region may be included at the N-terminus of a soluble RANKL, as may be all or a portion of the transmembrane and/or intracellular domains, provided that the resulting soluble RANKL is not membrane-associated. Accordingly, a soluble RANKL will have an N-terminal amino acid selected from the group consisting of amino acids 1 through 162 of SEQ ID NO:13 (1 through 139 of SEQ ID NO: 11). Preferably, the amino terminal amino acid is between amino acids 69 and 162 of SEQ ID NO. 13 (human RANKL; amino acids 48 and 139 of SEQ ID NO. 11). Similarly, the carboxy terminal amino acid can be between amino acid 313 and 317 of SEQ ID NO. 13 (human RANKL; corresponding to amino acids 290 through 294 of SEQ ID NO: 11). Those skilled in the art can prepare these and additional soluble forms through routine experimentation.

Fragments can be prepared using known techniques to isolate a desired portion of the extracellular region, and can be prepared, for example, by comparing the extracellular region with those of other members of the TNF family (of which RANKL is a member) and selecting forms similar to those prepared for other family members. Alternatively, unique restriction sites or PCR techniques that are known in the art can be used to prepare numerous truncated forms which can be expressed and analyzed for activity.

Other derivatives of the RANKL proteins within the scope of this invention include covalent or aggregative conjugates

of the proteins or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α -factor leader).

Protein fusions can comprise peptides added to facilitate purification or identification of RANKL proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as that described by Hopp et al., *Bio/Technology* 6:1204 (1988). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein. Fusion proteins capped with such peptides may also be resistant to intracellular degradation in *E. coli*.

Fusion proteins further comprise the amino acid sequence of a RANKL linked to an immunoglobulin Fc region. An exemplary Fc region is a human IgG₁ having a nucleotide an amino acid sequence set forth in SEQ ID NO: 8. Fragments of an Fc region may also be used, as can Fc muteins. For example, certain residues within the hinge region of an Fc region are critical for high affinity binding to Fc γ RI. Canfield and Morrison (*J. Exp. Med.* 173:1483; 1991) reported that Leu₍₂₃₄₎ and Leu₍₂₃₅₎ were critical to high affinity binding of IgG₁ to Fc γ RI present on U937 cells. Similar results were obtained by Lund et al. (*J. Immunol.* 147:2657, 1991; *Molecular Immunol.* 29:53, 1991). Such mutations, alone or in combination, can be made in an IgG₁ Fc region to decrease the affinity of IgG₁ for FcR. Depending on the portion of the Fc region used, a fusion protein may be expressed as a dimer, through formation of interchain disulfide bonds. If the fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a protein oligomer with as many as four RANKL regions.

In another embodiment, RANKL proteins further comprise an oligomerizing peptide such as a leucine zipper domain. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Leucine zipper domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for dimerization of the proteins. The leucine zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, with four or five leucine residues interspersed with other amino acids. Examples of leucine zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, fos and jun, also exhibit leucine zipper domains, as does the gene product of the murine proto-oncogene, c-myc (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes fos and jun comprise leucine zipper domains preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989). The leucine zipper domain is necessary for biological activity (DNA binding) in these proteins.

The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess leucine zipper domains

(Buckland and Wild, *Nature* 338:547, 1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990). The leucine zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the leucine zipper domains could contribute to the oligomeric structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al., *Proc. Natl. Acad. Sci. U.S.A.* 88:3523, 1991). Leucine zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science* 259:230, 1993).

Leucine zipper domains fold as short, parallel coiled coils. (O'Shea et al., *Science* 254:539; 1991) The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick in 1953 (*Acta Crystallogr.* 6:689). The dimer formed by a leucine zipper domain is stabilized by the heptad repeat, designated (abcdefg), according to the notation of McLachlan and Stewart (*J. Mol. Biol.* 98:293; 1975), in which residues a and d are generally hydrophobic residues, with d being a leucine, which line up on the same face of a helix. Oppositely-charged residues commonly occur at positions g and e. Thus, in a parallel coiled coil formed from two helical leucine zipper domains, the "knobs" formed by the hydrophobic side chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

The leucine residues at position d contribute large hydrophobic stabilization energies, and are important for dimer formation (Krystek et al., *Int. J. Peptide Res.* 38:229, 1991). Lovejoy et al. recently reported the synthesis of a triple-stranded a-helical bundle in which the helices run up-down (*Science* 259:1288, 1993). Their studies confirmed that hydrophobic stabilization energy provides the main driving force for the formation of coiled coils from helical monomers. These studies also indicate that electrostatic interactions contribute to the stoichiometry and geometry of coiled coils. Several studies have indicated that conservative amino acids may be substituted for individual leucine residues with minimal decrease in the ability to dimerize; multiple changes, however, usually result in loss of this ability (Landschulz et al., *Science* 243:1681, 1989; Turner and Tjian, *Science* 243:1689, 1989; Hu et al., *Science* 250:1400, 1990). van Heekeren et al. reported that a number of different amino residues can be substituted for the leucine residues in the leucine zipper domain of GCN4, and further found that some GCN4 proteins containing two leucine substitutions were weakly active (*Nucl. Acids Res.* 20:3721, 1992). Mutation of the first and second heptadic leucines of the leucine zipper domain of the measles virus fusion protein (MVF) did not affect syncytium formation (a measure of virally-induced cell fusion); however, mutation of all four leucine residues prevented fusion completely (Buckland et al., *J. Gen. Virol.* 73:1703, 1992). None of the mutations affected the ability of MVF to form a tetramer.

Amino acid substitutions in the a and d residues of a synthetic peptide representing the GCN4 leucine zipper domain have been found to change the oligomerization properties of the leucine zipper domain (Alber, Sixth Symposium of the Protein Society, San Diego, Calif.). When all residues at position a are changed to isoleucine, the leucine zipper still forms a parallel dimer. When, in addition to this change, all leucine residues at position d are also changed to isoleucine, the resultant peptide spontaneously forms a trimeric parallel coiled coil in solution. Substituting all

amino acids at position d with isoleucine and at position a with leucine results in a peptide that tetramerizes. Peptides containing these substitutions are still referred to as leucine zipper domains.

The present invention also includes RANKL with or without associated native-pattern glycosylation. Proteins expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of DNAs encoding the inventive proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of RANKL protein having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A₁-Z, where A₁ is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A₁ and Z, or an amino acid other than Asn between Asn and A₁.

RANKL protein derivatives may also be obtained by mutations of the native RANKL or subunits thereof. A RANKL mutated protein, as referred to herein, is a polypeptide homologous to a native RANKL protein, but which has an amino acid sequence different from the native protein because of one or a plurality of deletions, insertions or substitutions. The effect of any mutation made in a DNA encoding a mutated peptide may be easily determined by analyzing the ability of the mutated peptide to bind its counterstructure in a specific manner. Moreover, activity of RANKL analogs, muteins or derivatives can be determined by any of the assays described herein (for example, induction of NF- κ B activation).

Analogues of the inventive proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present.

When a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered. Subunits of the inventive proteins may be constructed by deleting terminal or internal residues or sequences. Soluble forms of RANKL can be readily prepared and tested for their ability to induce NF- κ B activation. Polypeptides corresponding to the cytoplasmic regions, and fragments thereof (for example, a death domain) can be prepared by similar techniques. Additional guidance as to the types of mutations that can be made is provided by a comparison of the sequence of RANKL to proteins that have similar structures, as well as by performing structural analysis of the inventive RANKL proteins.

Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those which do not affect the biological activity of RANKL (i.e., ability of the inventive proteins to bind antibodies to the corresponding native protein in substantially equivalent a

manner, the ability to bind the counterstructure in substantially the same manner as the native protein, the ability to induce a RANKL signal, or ability to induce NF- κ B activation). Examples of conservative substitutions include substitution of amino acids outside of the binding domain(s) (either ligand/receptor or antibody binding areas for the extracellular domain, or regions that interact with other, intracellular proteins for the cytoplasmic domain), and substitution of amino acids that do not alter the secondary and/or tertiary structure of the native protein. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Mutations in nucleotide sequences constructed for expression of analog proteins or fragments thereof must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins which would adversely affect translation of the mRNA.

Not all mutations in the nucleotide sequence which encodes a RANKL protein or fragments thereof will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

Although a mutation site may be predetermined, it is not necessary that the nature of the mutation per se be predetermined. For example, in order to select for optimum characteristics of mutants, random mutagenesis may be conducted and the expressed mutated proteins screened for the desired activity. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584 and 4,737,462 disclose suitable techniques, and are incorporated by reference herein.

Additional embodiments of the inventive proteins include RANKL polypeptides encoded by DNAs capable of hybridizing to the DNAs of SEQ ID NO: 10 or 12 under moderately stringent conditions (prewashing solution of 5 \times SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50 $^{\circ}$ C., 5 \times SSC, overnight) to the DNA sequences encoding RANKL, or more preferably under stringent conditions (for example, hybridization in 6 \times SSC at 63 $^{\circ}$ C. overnight; washing in 3 \times SSC at 55 $^{\circ}$ C.), and other sequences which are degenerate to those which encode the RANKL. In one embodiment, RANKL polypeptides are at least about 70% identical in amino acid sequence to the

amino acid sequence of native RANKL protein as set forth in SEQ ID NOs: 10 and 12. In a preferred embodiment, RANKL polypeptides are at least about 80% identical in amino acid sequence to the native form of RANKL; most preferred polypeptides are those that are at least about 90% identical to native RANKL.

Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWCGG). For fragments derived from the RANKL protein, the identity is calculated based on that portion of the RANKL protein that is present in the fragment.

The biological activity of RANKL analogs or muteins can be determined by testing the ability of the analogs or muteins to induce a signal through RANK, for example, activation of transcription as described in the Examples herein. Alternatively, suitable assays, for example, an enzyme immunoassay or a dot blot, employing an antibody that binds native RANKL, or a soluble form of RANK, can be used to assess the activity of RANKL analogs or muteins. Suitable assays also include, for example, assays that measure the ability of a RANKL peptide or mutein to bind cells expressing RANK, and/or the biological effects thereon. Such methods are well known in the art.

Fragments of the RANKL nucleotide sequences are also useful. In one embodiment, such fragments comprise at least about 17 consecutive nucleotides, preferably at least about 25 nucleotides, more preferably at least 30 consecutive nucleotides, of the RANKL DNA disclosed herein. DNA and RNA complements of such fragments are provided herein, along with both single-stranded and double-stranded forms of the RANKL DNAs of SEQ ID NOs: 10 and 12, and those encoding the aforementioned polypeptides. A fragment of RANKL DNA generally comprises at least about 17 nucleotides, preferably from about 17 to about 30 nucleotides. Such nucleic acid fragments (for example, a probe corresponding to the extracellular domain of RANKL) are used as a probe or as primers in a polymerase chain reaction (PCR).

The probes also find use in detecting the presence of RANKL nucleic acids in *in vitro* assays and in such procedures as Northern and Southern blots. Cell types expressing RANKL can be identified as well. Such procedures are well known, and the skilled artisan can choose a probe of suitable length, depending on the particular intended application. For PCR, 5' and 3' primers corresponding to the termini of a desired RANKL DNA sequence are employed to amplify that sequence, using conventional techniques.

Other useful fragments of the RANKL nucleic acids are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target RANKL mRNA (sense) or RANKL DNA (antisense) sequences. The ability to create an antisense or a sense oligonucleotide, based upon a cDNA sequence for a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659, 1988 and van der Krol et al., *BioTechniques* 6:958, 1988.

Uses of DNAs, Proteins and Analogs

The RANKL DNAs, proteins and analogs described herein will have numerous uses, including the preparation of pharmaceutical compositions. For example, soluble forms of RANKL will be useful to transduce signal via RANK. RANKL compositions (both protein and DNAs) will also be useful in development of antibodies to RANKL, both those that inhibit binding to RANK and those that do not. Also provided herein are compositions comprising antibodies to

RANKL to be used, for example, in interfering with RANKL signaling, as components of diagnostic or research assays for RANKL or RANKL activity, or in affinity purification of RANKL. The inventive DNAs are useful for the expression of recombinant proteins, and as probes for analysis (either quantitative or qualitative) of the presence or distribution of RANKL transcripts.

The inventive proteins will also be useful in preparing kits that are used to detect soluble RANK or RANKL, or monitor RANK-related activity, for example, in patient specimens. RANKL proteins will also find uses in monitoring RANK-related activity in other samples or compositions, as is necessary when screening for antagonists or mimetics of this activity (for example, peptides or small molecules that inhibit or mimic, respectively, the interaction). A variety of assay formats are useful in such kits, including (but not limited to) ELISA, dot blot, solid phase binding assays (such as those using a biosensor), rapid format assays and bioassays.

The purified RANKL according to the invention will facilitate the discovery of inhibitors of RANK, and thus, inhibitors of an inflammatory response (via inhibition of NF- κ B activation). The use of a purified RANKL polypeptide in the screening for potential inhibitors is important and can virtually eliminate the possibility of interfering reactions with contaminants. Such a screening assay can utilize either the extracellular domain of RANKL, or a fragment thereof. Detecting the inhibiting activity of a molecule would typically involve use of a soluble form of RANKL derived from the extracellular domain in a screening assay to detect molecules capable of binding RANK and inhibiting binding of the RANKL.

In addition, RANKL polypeptides can also be used for structure-based design of RANKL-inhibitors. Such structure-based design is also known as "rational drug design." The RANKL polypeptides can be three-dimensionally analyzed by, for example, X-ray crystallography, nuclear magnetic resonance or homology modeling, all of which are well-known methods. The use of RANKL structural information in molecular modeling software systems to assist in inhibitor design is also encompassed by the invention. Such computer-assisted modeling and drug design may utilize information such as chemical conformational analysis, electrostatic potential of the molecules, protein folding, etc. A particular method of the invention comprises analyzing the three dimensional structure of RANKL for likely binding sites of substrates, synthesizing a new molecule that incorporates a predictive reactive site, and assaying the new molecule as described above.

Moreover, as shown in the Examples herein, soluble forms of RANKL will be useful to induce maturation of dendritic cells (DC), and to enhance their allo-stimulatory capacity. Accordingly, RANKL proteins will be useful in augmenting an immune response, and can be used for these purposes either *ex vivo* (i.e., in obtaining cells such as DC from an individual, exposing them to antigen and cytokines *ex vivo*, and re-administering them to the individual) or *in vivo* (i.e., as a vaccine adjuvant that will augment humoral and/or cellular immunity). RANKL will also be useful promoting viability of T cells in the presence of TGF β , which will also be helpful in regulating an immune response. Expression of Recombinant RANKL

The proteins of the present invention are preferably produced by recombinant DNA methods by inserting a DNA sequence encoding RANKL protein or an analog thereof into a recombinant expression vector and expressing the DNA

sequence in a recombinant expression system under conditions promoting expression. DNA sequences encoding the proteins provided by this invention can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being inserted in a recombinant expression vector and expressed in a recombinant transcriptional unit.

Recombinant expression vectors include synthetic or cDNA-derived DNA fragments encoding RANKL, or homologs, muteins or bioequivalent analogs thereof, operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation, as described in detail below. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated.

DNA regions are operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. DNA sequences encoding RANKL, or homologs or analogs thereof which are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, Wis., USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the β -lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage λ P_L promoter and cI857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other

glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and α -factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). The yeast α -factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., *Cell* 30:933, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the Hind III site toward the Bgl II site located in the viral origin of replication is included. Further, viral genomic promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen. Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A preferred eukaryotic vector for expression of RANKL DNA is referred to as pDC406 (McMahan et al., *EMBO J.* 10:2821, 1991), and includes regulatory sequences derived from SV40, human immunodeficiency virus (HIV), and Epstein-Barr virus (EBV). Other preferred vectors include pDC409 and pDC410, which are derived from pDC406. pDC410 was derived from pDC406 by substituting the EBV origin of replication with sequences encoding the SV40 large T antigen. pDC409 differs from pDC406 in that a Bgl II restriction site outside of the multiple cloning site has been deleted, making the Bgl II site within the multiple cloning site unique.

A useful cell line that allows for episomal replication of expression vectors, such as pDC406 and pDC409, which contain the EBV origin of replication, is CV-1/EBNA (ATCC CRL 10478). The CV-1/EBNA cell line was derived by transfection of the CV-1 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) and consti-

tutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter.

Host Cells

Transformed host cells are cells which have been transformed or transfected with expression vectors constructed using recombinant DNA techniques and which contain sequences encoding the proteins of the present invention. Transformed host cells may express the desired protein (RANKL, or homologs or analogs thereof), but host cells transformed for purposes of cloning or amplifying the inventive DNA do not need to express the protein. Expressed proteins will preferably be secreted into the culture supernatant, depending on the DNA selected, but may be deposited in the cell membrane.

Suitable host cells for expression of proteins include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacillus* spp. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce proteins using RNAs derived from the DNA constructs disclosed herein. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, N.Y., 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotic expression hosts may be used for expression of RANKL, or homologs or analogs thereof that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Recombinant RANKL may also be expressed in yeast hosts, preferably from the *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2μ yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding the protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* *trp1* gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable yeast transformation protocols are known to those of skill in the art; an exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978, selecting for *Trp*⁺ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μ g/ml adenine and 20 μ g/ml uracil. Host strains transformed by vectors comprising the ADH2

promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μ g/ml adenine and 80 μ g/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4° C. prior to further purification.

Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, CV-1/EBNA (ATCC CRL 10478), L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

Purification of Recombinant RANKL

Purified RANKL, and homologs or analogs thereof are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a counter structure protein or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying RANKL and homologs thereof. For example, a RANKL expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a RANKL protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing zipper domain. Monoclonal antibodies against the RANKL protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand may also be used to prepare an affinity matrix for affinity purification of RANKL.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a RANKL composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, fol-

lowed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

Protein synthesized in recombinant culture is characterized by the presence of cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the inventive proteins free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

Uses and Administration of RANKL Compositions

The present invention provides methods of using therapeutic compositions comprising an effective amount of a protein and a suitable diluent and carrier, and methods for regulating an immune or inflammatory response. The use of RANKL in conjunction with soluble cytokine receptors or cytokines, or other immunoregulatory molecules is also contemplated.

For therapeutic use, purified protein is administered to a patient, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example, RANKL protein compositions administered to regulate immune function can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique. Typically, a therapeutic agent will be administered in the form of a composition comprising purified RANKL, in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages and concentrations employed.

Ordinarily, the preparation of such protein compositions entails combining the inventive protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and so forth.

As shown herein, RANKL has beneficial effects on various cells important in the immune system. Accordingly, RANKL may be administered to an individual as a vaccine adjuvant, or as a therapeutic agent to upregulate an immune response, for example, in infectious disease. Moreover, NF- κ B has

been found to play a protective role in preventing apoptotic death of cells induced by TNF- α or chemotherapy. Accordingly, agonists of RANK (i.e., RANKL and agonistic antibodies) will be useful in protecting RANK-expressing cells from the negative effects of chemotherapy or the presence of high levels of TNF- α such as occur in sepsis (see, i.e., Barinaga, *Science* 274 "724, 1996, and the articles by Beg and Baltimore and Wang et al., pages 782 and 784 of that same issue of *Science*).

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

EXAMPLE 1

The example describes the identification and isolation of a DNA encoding a novel member of the TNF receptor superfamily. A partial cDNA insert with a predicted open reading frame having some similarity to CD40 (a cell-surface antigen present on the surface of both normal and neoplastic human B cells that has been shown to play an important role in B-cell proliferation and differentiation; Stamenkovic et al., *EMBO J.* 8:1403, 1989), was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendritic cells (DC). The insert was excised from the vector by restriction endonuclease digestion, gel purified, labeled with 32 P, and used to hybridize to colony blots generated from a DC cDNA library containing larger cDNA inserts using high stringency hybridization and washing techniques (hybridization in 5 \times SSC, 50% formamide at 42 $^{\circ}$ C. overnight, washing in 0.5 \times SSC at 63 $^{\circ}$ C.); other suitable high stringency conditions are disclosed in Sambrook et al. in *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; 1989), 9.52-9.55. Initial experiments yielded a clone referred to as 9D-8A (SEQ ID NO: 1); subsequent analysis indicated that this clone contained all but the extreme 5' end of a novel cDNA, with predicted intron sequence at the extreme 5' end (nucleotides 1-92 of SEQ ID NO: 1). Additional colony hybridizations were performed, and a second clone was isolated. The second clone, referred to as 9D-15C (SEQ ID NO: 3), contained the 5' end without intron interruption but not the full 3' end. SEQ ID NO: 5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOs: 1 and 3.

The encoded protein was designated RANK, for receptor activator of NF- κ B. The cDNA encodes a predicted Type 1 transmembrane protein having 616 amino acid residues, with a predicted 24 amino acid signal sequence (the computer predicted cleavage site is after Leu24), a 188 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 383 amino acid cytoplasmic tail. The extracellular region of RANK displayed significant amino acid homology (38.5% identity, 52.3% similarity) to CD40. A cloning vector (pBluescriptSK³¹) containing human RANK sequence, designated pBluescript:huRANK (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, Md. (ATCC) on Dec. 20, 1996, under terms of the Budapest Treaty, and given accession number 98285.

EXAMPLE 2

This example describes construction of a RANK DNA construct to express a RANK/Fc fusion protein. A soluble

form of RANK fused to the Fc region of human IgG₁ was constructed in the mammalian expression vector pDC409 (U.S. Ser. No. 08/571,579). This expression vector encodes the leader sequence of the Cytomegalovirus (CMV) open reading frame R27080 (SEQ ID NO: 9), followed by amino acids 33–213 of RANK, followed by a mutated form of the constant domain of human IgG₁ that exhibits reduced affinity for Fc receptors (SEQ ID NO: 8; for the fusion protein, the Fc portion of the construct consisted of Arg3 through Lys232). An alternative expression vector encompassing amino acids 1–213 of RANK (using the native leader sequence) followed by the IgG₁ mutein was also prepared. Both expression vectors were found to induce high levels of expression of the RANK/Fc fusion protein in transfected cells.

To obtain RANK/Fc protein, a RANK/Fc expression plasmid is transfected into CV-1/EBNA cells, and supernatants are collected for about one week. The RANK/Fc fusion protein is purified by means well-known in the art for purification of Fc fusion proteins, for example, by protein A sepharose column chromatography according to manufacturer's recommendations (i.e., Pharmacia, Uppsala, Sweden). SDS-polyacrylamide gel electrophoresis analysis indicated that the purified RANK/Fc protein migrated with a molecular weight of ~55 kDa in the presence of a reducing agent, and at a molecular weight of ~110 kDa in the absence of a reducing agent.

N-terminal amino acid sequencing of the purified protein made using the CMV R27080 leader showed 60% cleavage after Ala20, 20% cleavage after Pro22 and 20% cleavage after Arg28 (which is the Furin cleavage site; amino acid residues are relative to SEQ ID NO: 9); N-terminal amino acid analysis of the fusion protein expressed with the native leader showed cleavage predominantly after Gln25 (80% after Gln25 and 20% after Arg23; amino acid residues are relative to SEQ ID NO: 6, full-length RANK). Both fusion proteins were able to bind a ligand for RANK in a specific manner (i.e., they bound to the surface of various cell lines such as a murine thymoma cell line, EL4), indicating that the presence of additional amino acids at the N-terminus of RANK does not interfere with its ability to bind RANKL. Moreover, the construct comprising the CMV leader encoded RANK beginning at amino acid 33; thus, a RANK peptide having an N-terminus at an amino acid between Arg23 and Pro33, inclusive, is expected to be able to bind a ligand for RANK in a specific manner.

Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a 'spacer' region, which is not necessary for ligand binding. In RANK, the amino acids between 196 and 213 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 213 and 196 of SEQ ID NO: 6, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 3

This example illustrates the preparation of monoclonal antibodies against RANK. Preparations of purified recombinant RANK, for example, or transfected cells expressing high levels of RANK, are employed to generate monoclonal antibodies against RANK using conventional techniques,

such as those disclosed in U.S. Pat. No. 4,411,993. DNA encoding RANK can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in interfering with RANK-induced signaling (antagonistic or blocking antibodies) or in inducing a signal by cross-linking RANK (agonistic antibodies), as components of diagnostic or research assays for RANK or RANK activity, or in affinity purification of RANK.

To immunize rodents, RANK immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, Mont.), and injected in amounts ranging from 10–100 µg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intramuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with RANK, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in U.S. Pat. No. 4,703,004. A preferred screening technique is the antibody capture technique described by Beckman et al., *J. Immunol.* 144:4212 (1990). Positive clones are then injected into the peritoneal cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-RANK monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to RANK protein.

Monoclonal antibodies were generated using RANK/Fc fusion protein as the immunogen. These reagents were screened to confirm reactivity against the RANK protein. Using the methods described herein to monitor the activity of the mAbs, both blocking (i.e., antibodies that bind RANK and inhibit binding of a ligand to RANK) and non-blocking (i.e., antibodies that bind RANK and do not inhibit ligand binding) were isolated.

EXAMPLE 4

This example illustrates the induction of NF-κB activity by RANK in 293/EBNA cells (cell line was derived by transfection of the 293 cell line with a gene encoding

Epstein-Barr virus nuclear antigen-1 (EBNA-1) that constitutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter). Activation of NF- κ B activity was measured in 293/EBNA cells essentially as described by Yao et al. (Immunity 3:811, 1995). Nuclear extracts were prepared and analyzed for NF- κ B activity by a gel retardation assay using a 25 base pair oligonucleotide spanning the NF- κ B binding sites. Two million cells were seeded into 10 cm dishes two days prior to DNA transfection and cultured in DMEM-F12 media containing 2.5% FBS (fetal bovine serum). DNA transfections were performed as described herein for the IL-8 promoter/reporter assays.

Nuclear extracts were prepared by solubilization of isolated nuclei with 400 mM NaCl (Yao et al., supra). Oligonucleotides containing an NF- κ B binding site were annealed and endlabeled with 32 P using T4 DNA polynucleotide kinase. Mobility shift reactions contained 10 μ g of nuclear extract, 4 μ g of poly(dI-dC) and 15,000 cpm labeled double-stranded oligonucleotide and incubated at room temperature for 20 minutes. Resulting protein-DNA complexes were resolved on a 6% native polyacrylamide gel in 0.25 \times Tris-borate-EDTA buffer.

Overexpression of RANK resulted in induction of NF- κ B activity as shown by an appropriate shift in the mobility of the radioactive probe on the gel. Similar results were observed when RANK was triggered by a ligand that binds RANK and transduces a signal to cells expressing the receptor (i.e., by co-transfecting cells with human RANK and murine RANKL DNA; see Example 7 below), and would be expected to occur when triggering is done with agonistic antibodies.

EXAMPLE 5

This example describes a gene promoter/reporter system based on the human Interleukin-8 (IL-8) promoter used to analyze the activation of gene transcription in vivo. The induction of human IL-8 gene transcription by the cytokines Interleukin-1 (IL-1) or tumor necrosis factor- α (TNF- α) is known to be dependent upon intact NF- κ B and NF-IL-6 transcription factor binding sites. Fusion of the cytokine-responsive IL-8 promoter with a cDNA encoding the murine IL-4 receptor (mIL-4R) allows measurement of promoter activation by detection of the heterologous reporter protein (mIL-4R) on the cell surface of transfected cells.

Human kidney epithelial cells (293/EBNA) are transfected (via the DEAE/DEXTRAN method) with plasmids encoding: 1) the reporter/promoter construct (referred to as pIL-8rep), and 2) the cDNA(s) of interest. DNA concentrations are always kept constant by the addition of empty vector DNA. The 293/EBNA cells are plated at a density of 2.5×10^4 cells/ml (3 ml/well) in a 6 well plate and incubated for two days prior to transfection. Two days after transfection, the mIL-4 receptor is detected by a radioimmunoassay (RIA) described below.

In one such experiment, the 293/EBNA cells were co-transfected with DNA encoding RANK and with DNA encoding RANKL (see Example 7 below). Co-expression of this receptor and its counterstructure by cells results in activation of the signaling process of RANK. For such co-transfection studies, the DNA concentration/well for the DEAE transfection were as follows: 40 ng of pIL-8rep [pBluescriptSK⁻ vector (Stratagene)]; 0.4 ng CD40 (DNA encoding CD40, a control receptor; pCDM8 vector); 0.4 ng RANK (DNA encoding RANK; pDC409 vector), and either 1–50 ng CD40L (DNA encoding the ligand for CD40, which acts as a positive control when co-transfected with CD40

and as a negative control when co-transfected with RANK; in pDC304) or RANKL (DNA encoding a ligand for RANK; in pDC406). Similar experiments can be done using soluble RANKL or agonistic antibodies to RANK to trigger cells transfected with RANK.

For the mIL-4R-specific RIA, a monoclonal antibody reactive with mIL-4R is labeled with 125 I via a Chloramine T conjugation method; the resulting specific activity is typically 1.5×10^{16} cpm/nmol. After 48 hours, transfected cells are washed once with media (DMEM/F12 5% FBS). Non-specific binding sites are blocked by the addition of pre-warmed binding media containing 5% non-fat dry milk and incubation at 37° C./5% CO₂ in a tissue culture incubator for one hour. The blocking media is decanted and binding buffer containing 125 I anti-mIL-4R (clone M1; rat IgG1) is added to the cells and incubated with rocking at room temperature for 1 hour. After incubation of the cells with the radio-labeled antibody, cells are washed extensively with binding buffer (2 \times) and twice with phosphate-buffered saline (PBS). Cells are lysed in 1 ml of 0.5 M NaOH, and total radioactivity is measured with a gamma counter.

Using this assay, 293/EBNA co-transfected with DNAs encoding RANK demonstrated transcriptional activation, as shown by detection of mIL-4R on the cell surface. Overexpression of RANK resulted in transcription of mIL-4R, as did triggering of the RANK by RANKL. Similar results are observed when RANK is triggered by agonistic antibodies.

EXAMPLE 6

This example illustrates the association of RANK with TRAF proteins. Interaction of RANK with cytoplasmic TRAF proteins was demonstrated by co-immunoprecipitation assays essentially as described by Hsu et al. (*Cell* 84:299; 1996). Briefly, 293/EBNA cells were co-transfected with plasmids that direct the synthesis of RANK and epitope-tagged (FLAG®; SEQ ID NO: 7) TRAF2 or TRAF3. Two days after transfection, surface proteins were labeled with biotin-ester, and cells were lysed in a buffer containing 0.5% NP-40. RANK and proteins associated with this receptor were immunoprecipitated with anti-RANK, washed extensively, resolved by electrophoretic separation on a 6–10% SDS polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane for Western blotting. The association of TRAF2 and TRAF3 proteins with RANK was visualized by probing the membrane with an antibody that specifically recognizes the FLAG® epitope. TRAFs 2 and 3 did not immunoprecipitate with anti-RANK in the absence of RANK expression.

EXAMPLE 7

This example describes isolation of a ligand for RANK, referred to as RANKL, by direct expression cloning. The ligand was cloned essentially as described in U.S. Ser. No. 08/249,189, filed May 24, 1994 (the relevant disclosure of which is incorporated by reference herein), for CD40L. Briefly, a library was prepared from a clone of a mouse thymoma cell line EL-4 (ATCC TIB 39), called EL-40.5, derived by sorting five times with biotinylated CD40/Fc fusion protein in a FACS (fluorescence activated cell sorter). The cDNA library was made using standard methodology; the plasmid DNA was isolated and transfected into sub-confluent CV1-EBNA cells using a DEAE-dextran method. Transfectants were screened by slide autoradiography for expression of RANKL using a two-step binding method with RANK/Fc fusion protein as prepared in Example 2 followed by radioiodinated goat anti-human IgG antibody.

A clone encoding a protein that specifically bound RANK was isolated and sequenced; the clone was referred to as 11H. An expression vector containing murine RANKL sequence, designated pDC406:muRANK-L (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, Md. (ATCC) on Dec. 20, 1996, under terms of the Budapest Treaty, and given accession number 98284. The nucleotide sequence and predicted amino acid sequence of this clone are illustrated in SEQ ID NO: 10. This clone did not contain an initiator methionine; additional, full-length clones were obtained from a 7B9 library (prepared substantially as described in U.S. Pat. No. 5,599,905, issued Feb. 4, 1997); the 5' region was found to be identical to that of human RANKL as shown in SEQ ID NO: 12, amino acids 1 through 22, except for substitution of a Gly for a Thr at residue 9.

This ligand is useful for assessing the ability of RANK to bind RANKL by a number of different assays. For example, transfected cells expressing RANKL can be used in a FACS assay (or similar assay) to evaluate the ability of soluble RANK to bind RANKL. Moreover, soluble forms of RANKL can be prepared and used in assays that are known in the art (i.e., ELISA or BIAcore assays essentially as described in U.S. Ser. No. 08/249,189, filed May 24, 1994). RANKL is also useful in affinity purification of RANK, and as a reagent in methods to measure the levels of RANK in a sample. Soluble RANKL is also useful in inducing NF- κ B activation and thus protecting cells that express RANK from apoptosis.

EXAMPLE 8

This example describes the isolation of a human RANK ligand (RANKL) using a PCR-based technique. Murine RANK ligand-specific oligonucleotide primers were used in PCR reactions using human cell line-derived first strand cDNAs as templates. Primers corresponded to nucleotides 478-497 and to the complement of nucleotides 858-878 of murine RANK ligand (SEQ ID NO: 10). An amplified band approximately 400 bp in length from one reaction using the human epidermoid cell line KB (ATCC CCL-17) was gel purified, and its nucleotide sequence determined; the sequence was 85% identical to the corresponding region of murine RANK ligand, confirming that the fragment was from human RANKL.

To obtain full-length human RANKL cDNAs, two human RANKL-specific oligonucleotides derived from the KB PCR product nucleotide sequence were radiolabeled and used as hybridization probes to screen a human PBL cDNA library prepared in lambda gt10 (Stratagene, La Jolla, Calif.), substantially as described in U.S. Pat. No. 5,599,905, issued Feb. 4, 1997. Several positive hybridizing plaques were identified and purified, their inserts subcloned into pBluescript SK⁻ (Stratagene, La Jolla, Calif.), and their nucleotide sequence determined. One isolate, PBL3, was found to encode most of the predicted human RANKL, but appeared to be missing approximately 200 bp of 5' coding region. A second isolate, PBL5 was found to encode much of the predicted human RANKL, including the entire 5' end and an additional 200 bp of 5' untranslated sequence.

The 5' end of PBL5 and the 3' end of PBL3 were ligated together to form a full length cDNA encoding human RANKL. The nucleotide and predicted amino acid sequence of the full-length human RANK ligand is shown in SEQ ID NO: 12. Human RANK ligand shares 83% nucleotide and 84% amino acid identity with murine RANK ligand. A plasmid vector containing human RANKL sequence, desig-

nated pBluescript:huRANK-L (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, Md. (ATCC) on Mar. 11, 1997 under terms of the Budapest Treaty, and given accession number 98354.

Murine and human RANKL are Type 2 transmembrane proteins. Murine RANKL contains a predicted 48 amino acid intracellular domain, 21 amino acid transmembrane domain and 247 amino acid extracellular domain. Human RANKL contains a predicted 47 amino acid intracellular domain, 21 amino acid transmembrane domain and 249 amino acid extracellular domain.

EXAMPLE 9

This example describes the chromosomal mapping of human RANK using PCR-based mapping strategies. Initial human chromosomal assignments were made using RANK and RANKL-specific PCR primers and a BIOS Somatic Cell Hybrid PCRable DNA kit from BIOS Laboratories (New Haven, Conn.), following the manufacturer's instructions. RANK mapped to human chromosome 18; RANK ligand mapped to human chromosome 13. More detailed mapping was performed using a radiation hybrid mapping panel Genebridge 4 Radiation Hybrid Panel (Research Genetics, Huntsville, Ala.; described in Walter, M A et al., *Nature Genetics* 7:22-28, 1994). Data from this analysis was then submitted electronically to the MIT Radiation Hybrid Mapper (URL: <http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>) following the instructions contained therein. This analysis yielded specific genetic marker names which, when submitted electronically to the NCBI Entrez browser (URL: <http://www3.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=c&form=0>), yielded the specific map locations. RANK mapped to chromosome 18q22.1, and RANKL mapped to chromosome 13q14.

EXAMPLE 10

This example illustrates the preparation of monoclonal antibodies against RANKL. Preparations of purified recombinant RANKL, for example, or transfected cells expressing high levels of RANKL, are employed to generate monoclonal antibodies against RANKL using conventional techniques, such as those disclosed in U.S. Pat. No. 4,411,993. DNA encoding RANKL can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in interfering with RANKL signaling (antagonistic or blocking antibodies), as components of diagnostic or research assays for RANKL or RANK activity, or in affinity purification of RANKL.

To immunize rodents, RANKL immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, Mont.), and injected in amounts ranging from 10-100 μ g subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intramuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosor-

bent assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with RANKL, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in U.S. Pat. No. 4,703,004. A preferred screening technique is the antibody capture technique described by Beckman et al., *J. Immunol.* 144:4212 (1990). Positive clones are then injected into the peritoneal cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-RANK monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to RANKL protein. Using the methods described herein to monitor the activity of the mAbs, both blocking (i.e., antibodies that bind RANKL and inhibit binding to RANK) and non-blocking (i.e., antibodies that bind RANKL and do not inhibit binding) are isolated.

EXAMPLE 11

This example demonstrates that RANK expression can be up-regulated. Human peripheral blood T cells were purified by flow cytometry sorting or by negative selection using antibody coated beads, and activated with anti-CD3 (OKT3, Dako) coated plates or phytohemagglutinin in the presence or absence of various cytokines, including Interleukin-4 (IL-4), Transforming Growth Factor- β (TGF- β) and other commercially available cytokines (IL-1- α , IL-2, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IFN- γ , TNF- α). Expression of RANK was evaluated by FACS in a time course experiment for day 2 to day 8, using a mouse monoclonal antibody mAb144 (prepared as described in Example 3), as shown in the table below. Results are expressed as '+' to '++++' referring to the relative increase in intensity of staining with anti-RANK. Double labeling experiments using both anti-RANK and anti-CD8 or anti-CD4 antibodies were also performed.

TABLE 1

Upregulation of RANK by Cytokines	
Cytokine (concentration)	Results:
IL-4 (50 ng/ml)	+
TGF- β (5 ng/ml)	+ to ++
IL-4 (50 ng/ml) + TGF- β (5 ng/ml)	++++
IL-1- α (10 ng/ml)	—
IL-2 (20 ng/ml)	—
IL-3 (25 ng/ml)	—
IL-7 (20 ng/ml)	—
IL-8 (10 ng/ml)	—
IL-10 (50 ng/ml)	—
IL-12 (10 ng/ml)	—
IL-15 (10 ng/ml)	—

TABLE 1-continued

Upregulation of RANK by Cytokines	
Cytokine (concentration)	Results:
IFN- γ (100 U/ml)	—
TNF- α (10 ng/ml)	—

Of the cytokines tested, IL-4 and TGF- β increased the level of RANK expression on both CD8+ cytotoxic and CD4+ helper T cells from day 4 to day 8. The combination of IL-4 and TGF- β acted synergistically to upregulate expression of this receptor on activated T cells. This particular combination of cytokines is secreted by suppressor T cells, and is believed to be important in the generation of tolerance (reviewed in Mitchison and Sieper, *Z. Rheumatol.* 54:141, 1995), implicating the interaction of RANK in regulation of an immune response towards either tolerance or induction of an active immune response.

EXAMPLE 12

This example illustrates the influence of RANK.Fc and hRANKL on activated T cell growth. The addition of TGF β to anti-CD3 activated human peripheral blood T lymphocytes induces proliferation arrest and ultimately death of most lymphocytes within the first few days of culture. We tested the effect of RANK:RANKL interactions on TGF β -treated T cells by adding RANK.Fc or soluble human RANKL to T cell cultures.

Human peripheral blood T cells (7×10^5 PBT) were cultured for six days on anti-CD3 (OKT3, 5 μ g/ml) and anti-Flag (M1, 5 μ g/ml) coated 24 well plates in the presence of TGF β (1 μ g/ml) and IL4 (10 g/ml), with or without recombinant FLAG-tagged soluble hRANKL (1 μ g/ml) or RANK.Fc (10 μ g/ml). Viable T cell recovery was determined by triplicate trypan blue countings.

The addition of RANK.Fc significantly reduced the number of viable T cells recovered after six days, whereas soluble RANKL greatly increased the recovery of viable T cells (FIG. 1). Thus, endogenous or exogenous RANKL enhances the number of viable T cells generated in the presence of TGF β . TGF β , along with IL-4, has been implicated in immune response regulation when secreted by the T_H3 /regulatory T cell subset. These T cells are believed to mediate bystander suppression of effector T cells. Accordingly, RANK and its ligand may act in an auto/paracrine fashion to influence T cell tolerance. Moreover, TGF β is known to play a role in the evasion of the immune system effected by certain pathogenic or opportunistic organisms. In addition to playing a role in the development of tolerance, RANK may also play a role in immune system evasion by pathogens.

EXAMPLE 13

This example illustrates the influence of the interaction of RANK on CD1a+ dendritic cells (DC). Functionally mature dendritic cells (DC) were generated in vitro from CD34+ bone marrow (BM) progenitors. Briefly, human BM cells from normal healthy volunteers were density fractionated using Ficoll medium and CD34+ cells immunoaffinity isolated using an anti-CD34 matrix column (Ceprate, CellPro). The CD34+ BM cells were then cultured in human GM-CSF (20 ng/ml), human IL-4 (20 ng/ml), human TNF- α (20 ng/ml), human CHO-derived Flt3L (FL; 100 ng/ml) in Super McCoy's medium supplemented with 10% fetal calf serum

in a fully humidified 37° C. incubator (5% CO₂) for 14 days. CD1a⁺, HLA-DR⁺ DC were then sorted using a FACStar Plus™, and used for biological evaluation of RANK

On human CD1a⁺ DC derived from CD34⁺ bone marrow cells, only a subset (20–30%) of CD1a⁺ DC expressed RANK at the cell surface as assessed by flow cytometric analysis. However, addition of CD40L to the DC cultures resulted in RANK surface expression on the majority of CD1a⁺ DC. CD40L has been shown to activate DC by enhancing in vitro cluster formation, inducing DC morphological changes and upregulating HLA-DR, CD54, CD58, CD80 and CD86 expression

Addition of RANKL to DC cultures significantly increased the degree of DC aggregation and cluster formation above control cultures, similar to the effects seen with CD40L. Sorted human CD1a⁺ DC were cultured in a cytokine cocktail (GM-CSF, IL-4, TNF- α and FL) in cocktail plus CD40L (1 μ g/ml), in cocktail plus RANKL (1 μ g/ml), or in cocktail plus heat inactivated (Δ H) RANKL (1 μ g/ml) in 24-well flat bottomed culture plates in 1 ml culture media for 48–72 hours and then photographed using an inversion microscope. An increase in DC aggregation and cluster formation above control cultures was not evident when heat inactivated RANKL was used, indicating that this effect was dependent on biologically active protein. However, initial phenotypic analysis of adhesion molecule expression indicated that RANKL-induced clustering was not due to increased levels of CD2, CD11 a, CD54 or CD58.

The addition of RANKL to CD1a⁺ DC enhanced their allo-stimulatory capacity in a mixed lymphocyte reaction (MLR) by at least 3- to 10-fold, comparable to CD40L-cultured DC (FIG. 2). Allogeneic T cells (1 \times 10⁵) were incubated with varying numbers of irradiated (2000 rad) DC cultured as indicated above in 96-well round bottomed culture plates in 0.2 ml culture medium for four days. The cultures were pulsed with 0.5 mCi [³H]-thymidine for eight hours and the cells harvested onto glass fiber sheets for counting on a gas phase β counter. The background counts for either T cells or DC cultured alone were <100 cpm. Values represent the mean \pm SD of triplicate cultures. Heat inactivated RANKL had no effect. DC allo-stimulatory activity was not further enhanced when RANKL and CD40L were used in combination, possibly due to DC functional capacity having reached a maximal level with either cytokine alone. Neither RANKL nor CD40L enhanced the in vitro growth of DC over the three day culture period. Unlike CD40L, RANKL did not significantly increase the levels of HLA-DR expression nor the expression of CD80 or CD86.

RANKL can enhance DC cluster formation and functional capacity without modulating known molecules involved in cell adhesion (CD18, CD54), antigen presentation (HLA-DR) or costimulation (CD86), all of which are regulated by CD40/CD40L signaling. The lack of an effect on the expression of these molecules suggests that RANKL may regulate DC function via an alternate pathway(s) distinct from CD40/CD40L. Given that CD40L regulates RANK surface expression on in vitro-generated DC and that CD40L is upregulated on activated T cells during DC-T cell interactions, RANK and its ligand may form an important part of the activation cascade that is induced during DC-mediated T cell expansion. Furthermore, culture of DC in RANKL results in decreased levels of CD1b/c expression, and increased levels of CD83. Both of these molecules are similarly modulated during DC maturation by CD40L (Caux et al. *J. Exp. Med.* 180:1263; 1994), indicating that RANKL induces DC maturation. Dendritic cells are referred to as “professional” antigen presenting cells, and have a high capacity for

sensitizing MHC-restricted T cells. There is growing interest in using dendritic cells ex vivo as tumor or infectious disease vaccine adjuvants (see, for example, Romani, et al., *J. Exp. Med.*, 180:83, 1994). Therefore, an agent such as RANKL that induces DC maturation and enhances the ability of dendritic cells to stimulate an immune response is likely to be useful in immunotherapy of various diseases.

EXAMPLE 14

This example describes the isolation of the murine homolog of RANK, referred to as muRANK. MuRANK was isolated by a combination of cross-species PCR and colony hybridization. The conservation of Cys residues in the Cys-rich pseudorepeats of the extracellular domains of TNFR superfamily member proteins was exploited to design human RANK-based PCR primers to be used on murine first strand cDNAs from various sources. Both the sense upstream primer and the antisense downstream primer were designed to have their 3' ends terminate within Cys residues.

The upstream sense primer encoded nucleotides 272–295 of SEQ ID NO: 5 (region encoding amino acids 79–86); the downstream antisense primer encoded the complement of nucleotides 409–427 (region encoding amino acids 124–130). Standard PCR reactions were set up and run, using these primers and first strand cDNAs from various murine cell line or tissue sources. Thirty reaction cycles of 94° C. for 30 seconds, 50° C. for 30 seconds, and 72° C. for 20 seconds were run. PCR products were analyzed by electrophoresis, and specific bands were seen in several samples. The band from one sample was gel purified and DNA sequencing revealed that the sequence between the primers was approximately 85% identical to the corresponding human RANK nucleotide sequence.

A plasmid based cDNA library prepared from the murine fetal liver epithelium line FLE18 (one of the cell lines identified as positive in the PCR screen) was screened for full-length RANK cDNAs using murine RANK-specific oligonucleotide probes derived from the murine RANK sequence determined from sequencing the PCR product. Two cDNAs, one encoding the 5' end and one encoding the 3' end of full-length murine RANK (based on sequence comparison with the full-length human RANK) were recombined to generate a full-length murine RANK cDNA. The nucleotide and amino acid sequence of muRANK are shown in SEQ ID Nos: 14 and 15.

The cDNA encodes a predicted Type 1 transmembrane protein having 625 amino acid residues, with a predicted 30 amino acid signal sequence, a 184 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 390 amino acid cytoplasmic tail. The extracellular region of muRANK displayed significant amino acid homology (69.7% identity, 80.8% similarity) to huRANK. Those of skill in the art will recognize that the actual cleavage site can be different from that predicted by computer; accordingly, the N-terminal of RANK may be from amino acid 25 to amino acid 35.

Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a ‘spacer’ region, which is not necessary for ligand binding. In muRANK, the amino acids between 197 and 214 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the

group consisting of amino acids 214, and 197 of SEQ ID NO: 14, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 15

This example illustrates the preparation of several different soluble forms of RANK and RANKL. Standard techniques of restriction enzyme cutting and ligation, in combination with PCR-based isolation of fragments for which no convenient restriction sites existed, were used. When PCR was utilized, PCR products were sequenced to ascertain whether any mutations had been introduced; no such mutations were found.

In addition to the huRANK/Fc described in Example 2, another RANK/Fc fusion protein was prepared by ligating DNA encoding amino acids 1–213 of SEQ ID NO: 6, to DNA encoding amino acids 3–232 of the Fc mutein described previously (SEQ ID NO: 8). A similar construct was prepared for murine RANK, ligating DNA encoding amino acids 1–213 of full-length murine RANK (SEQ ID NO: 15) to DNA encoding amino acids 3–232 of the Fc mutein (SEQ ID NO: 8).

A soluble, tagged, poly-His version of huRANKL was prepared by ligating DNA encoding the leader peptide from the immunoglobulin kappa chain (SEQ ID NO: 16) to DNA encoding a short version of the FLAG™ tag (SEQ ID NO: 17), followed by codons encoding Gly Ser, then a poly-His tag (SEQ ID NO: 18), followed by codons encoding Gly Thr Ser, and DNA encoding amino acids 138–317 of SEQ ID NO: 13. A soluble, poly-His tagged version of murine RANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO: 9) to codons encoding Arg Thr Ser, followed by DNA encoding poly-His (SEQ ID NO: 18) followed by DNA encoding amino acids 119–294 of SEQ ID NO: 11.

A soluble, oligomeric form of huRANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO: 9) to a codon encoding Asp followed by DNA ending a trimer-former “leucine” zipper (SEQ ID NO: 19), then by codons encoding Thr Arg Ser followed by amino acids 138–317 of SEQ ID NO: 13.

These and other constructs are prepared by routine experimentation. The various DNAs are then inserted into a suitable expression vector, and expressed. Particularly preferred expression vectors are those which can be used in mammalian cells. For example, pDC409 and pDC304, described herein, are useful for transient expression. For stable transfection, the use of CHO cells is preferred; several useful vectors are described in U.S. Ser. No. 08/785,150, now allowed, for example, one of the 2A5–3 λ -derived expression vectors discussed therein.

EXAMPLE 16

This example demonstrates that RANKL expression can be up-regulated on murine T cells. Cells were obtained from mesenteric lymph nodes of C57BU6 mice, and activated with anti-CD3 coated plates, Concanavalin A (ConA) or phorbol myristate acetate in combination with ionomycin (anti-CD3: 500A2; Immunex Corporation, Seattle Wash.; ConA, PMA, ionomycin, Sigma, St. Louis, Mo.) substantially as described herein, and cultured from about 2 to 5 days. Expression of RANKL was evaluated in a three color analysis by FACS, using antibodies to the T cell markers CD4, CD8 and CD45RB, and RANK/Fc, prepared as described herein.

RANKL was not expressed on unstimulated murine T cells. T cells stimulated with either anti-CD3, ConA, or

PMA/ionomycin, showed differential expression of RANKL: CD4⁺/CD45RB^{Lo} and CD4⁺/CD45RB^{Hi} cells were positive for RANKL, but CD8⁺ cells were not. RANKL was not observed on B cells, similar to results observed with human cells.

EXAMPLE 17

This example illustrates the effects of murine RANKL on cell proliferation and activation. Various cells or cell lines representative of cells that play a role in an immune response (murine spleen, thymus and lymphnode) were evaluated by culturing them under conditions promoting their viability, in the presence or absence of RANKL. RANKL did not stimulate any of the tested cells to proliferate. One cell line, a macrophage cell line referred to as RAW 264.7 (ATCC accession number TIB 71) exhibited some signs of activation.

RAW cells constitutively produce small amounts of TNF- α . Incubation with either human or murine RANKL enhanced production of TNF- α by these cells in a dose dependent manner. The results were not due to contamination of RANKL preparations with endotoxin, since boiling RANKL for 10 minutes abrogated TNF- α production, whereas a similar treatment of purified endotoxin (LPS) did not affect the ability of the LPS to stimulate TNF- α production. Despite the fact that RANKL activated the macrophage cell line RAW T64.7 for TNF- α production, neither human RANKL nor murine RANKL stimulated nitric oxide production by these cells.

EXAMPLE 18

This example illustrates the effects of murine RANKL on growth and development of the thymus in fetal mice. Pregnant mice were injected with 1 mg of RANK/Fc or vehicle control protein (murine serum albumin; MSA) on days 13, 16 and 19 of gestation. After birth, the neonates continued to be injected with RANK/Fc intraperitoneally (IP) on a daily basis, beginning at a dose of 1 μ g, and doubling the dose about every four days, for a final dosage of 4 μ g. Neonates were taken at days 1, 8 and 15 post birth, their thymuses and spleens harvested and examined for size, cellularity and phenotypic composition.

A slight reduction in thymic size at day 1 was observed in the neonates born to the female injected with RANK/Fc; a similar decrease in size was not observed in the control neonates. At day 8, thymic size and cellularity were reduced by about 50% in the RANK/Fc-treated animals as compared to MSA treated mice. Phenotypic analysis demonstrated that the relative proportions of different T cell populations in the thymus were the same in the RANK/Fc mice as the control mice, indicating that the decreased cellularity was due to a global depression in the number of thymic T cells as opposed to a decrease in a specific population(s). The RANK/Fc-treated neonates were not significantly different from the control neonates at day 15 with respect to either size, cellularity or phenotype of thymic cells. No significant differences were observed in spleen size, cellularity or composition at any of the time points evaluated. The difference in cellularity on day 8 and not on day 15 may suggest that RANK/Fc may assert its effect early in thymic development.

EXAMPLE 19

This example demonstrates that the C-terminal region of the cytoplasmic domain of RANK is important for binding of several different TRAF proteins. RANK contains at least

two recognizable PXQX(X)T motifs that are likely TRAF docking sites. Accordingly, the importance of various regions of the cytoplasmic domain of RANK for TRAF binding was evaluated. A RANK/GST fusion protein was prepared substantially as described in Smith and Johnson, *Gene* 67:31 (1988), and used in the preparation of various truncations as described below.

Comparison of the nucleotide sequence of murine and human RANK indicated that there were several conserved regions that could be important for TRAF binding. Accordingly, a PCR-based technique was developed to facilitate preparation of various C-terminal truncations that would retain the conserved regions. PCR primers were designed to introduce a stop codon and restriction enzyme site at selected points, yielding the truncations described in Table 2 below. Sequencing confirmed that no undesired mutations had been introduced in the constructs.

Radio-labeled (^{35}S -Met, Cys) TRAF proteins were prepared by in vitro translation using a commercially available reticulocyte lysate kit according to manufacturer's instructions (Promega). Truncated GST fusion proteins were purified substantially as described in Smith and Johnson (supra). Briefly, *E. coli* were transfected with an expression vector encoding a fusion protein, and induced to express the protein. The bacteria were lysed, insoluble material removed, and the fusion protein isolated by precipitation with glutathione-coated beads (Sepahrose 4B, Pharmacia, Uppsala Sweden)

The beads were washed, and incubated with various radiolabeled TRAF proteins. After incubation and wash steps, the fusion protein/TRAF complexes were removed from the beads by boiling in 0.1% SDS+ β -mercaptoethanol, and loaded onto 12% SDS gels (Novex). The gels were subjected to autoradiography, and the presence or absence of radiolabeled material recorded. The results are shown in Table 2 below.

TABLE 2

Binding of Various TRAF Proteins to the Cytoplasmic Domain of RANK					
C terminal Truncations:	E206- S339	E206- Y421	E206- M476	E206- G544	Full length
TRAF1	-	-	-	-	++
TRAF2	-	-	-	-	++
TRAF3	-	-	-	-	++
TRAF4	-	-	-	-	-
TRAF5	-	-	-	-	+
TRAF6	-	+	+	+	++

These results indicate that TRAF1, TRAF2, TRAF3, TRAF 5 and TRAF6 bind to the most distal portion of the RANK cytoplasmic domain (between amino-acid G544 and A616). TRAF6 also has a binding site between S339 and Y421. In this experiment, TRAF5 also bound the cytoplasmic domain of RANK.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 19

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3115 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
(B) CLONE: 9D-8A

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 93..1868

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GCTGCTGCTG CTCTGCGCGC TGCTGCCCCG GCTGCAGTTT TATCCAGAAA GAGCTGTGTG      60
GACTCTCTGC CTGACCTCAG TGTTCCTTTC AG GTG GCT TTG CAG ATC GCT CCT      113
                               Val Ala Leu Gln Ile Ala Pro
                               1                               5
CCA TGT ACC AGT GAG AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC      161

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-continued

Pro	Cys	Thr	Ser	Glu	Lys	His	Tyr	Glu	His	Leu	Gly	Arg	Cys	Cys	Asn	
	10						15				20					
AAA	TGT	GAA	CCA	GGA	AAG	TAC	ATG	TCT	TCT	AAA	TGC	ACT	ACT	ACC	TCT	209
Lys	Cys	Glu	Pro	Gly	Lys	Tyr	Met	Ser	Ser	Lys	Cys	Thr	Thr	Thr	Ser	
	25					30					35					
GAC	AGT	GTA	TGT	CTG	CCC	TGT	GGC	CCG	GAT	GAA	TAC	TTG	GAT	AGC	TGG	257
Asp	Ser	Val	Cys	Leu	Pro	Cys	Gly	Pro	Asp	Glu	Tyr	Leu	Asp	Ser	Trp	
	40				45					50					55	
AAT	GAA	GAA	GAT	AAA	TGC	TTG	CTG	CAT	AAA	GTT	TGT	GAT	ACA	GGC	AAG	305
Asn	Glu	Glu	Asp	Lys	Cys	Leu	Leu	His	Lys	Val	Cys	Asp	Thr	Gly	Lys	
				60					65					70		
GCC	CTG	GTG	GCC	GTG	GTC	GCC	GGC	AAC	AGC	ACG	ACC	CCC	CGG	CGC	TGC	353
Ala	Leu	Val	Ala	Val	Val	Ala	Gly	Asn	Ser	Thr	Thr	Pro	Arg	Arg	Cys	
			75					80						85		
GCG	TGC	ACG	GCT	GGG	TAC	CAC	TGG	AGC	CAG	GAC	TGC	GAG	TGC	TGC	CGC	401
Ala	Cys	Thr	Ala	Gly	Tyr	His	Trp	Ser	Gln	Asp	Cys	Glu	Cys	Cys	Arg	
	90						95					100				
CGC	AAC	ACC	GAG	TGC	GCG	CCG	GGC	CTG	GGC	GCC	CAG	CAC	CCG	TTG	CAG	449
Arg	Asn	Thr	Glu	Cys	Ala	Pro	Gly	Leu	Gly	Ala	Gln	His	Pro	Leu	Gln	
	105					110					115					
CTC	AAC	AAG	GAC	ACA	GTG	TGC	AAA	CCT	TGC	CTT	GCA	GGC	TAC	TTC	TCT	497
Leu	Asn	Lys	Asp	Thr	Val	Cys	Lys	Pro	Cys	Leu	Ala	Gly	Tyr	Phe	Ser	
	120				125					130					135	
GAT	GCC	TTT	TCC	TCC	ACG	GAC	AAA	TGC	AGA	CCC	TGG	ACC	AAC	TGT	ACC	545
Asp	Ala	Phe	Ser	Ser	Thr	Asp	Lys	Cys	Arg	Pro	Trp	Thr	Asn	Cys	Thr	
			140						145					150		
TTC	CTT	GGA	AAG	AGA	GTA	GAA	CAT	CAT	GGG	ACA	GAG	AAA	TCC	GAT	GCG	593
Phe	Leu	Gly	Lys	Arg	Val	Glu	His	His	Gly	Thr	Glu	Lys	Ser	Asp	Ala	
			155					160						165		
GTT	TGC	AGT	TCT	TCT	CTG	CCA	GCT	AGA	AAA	CCA	CCA	AAT	GAA	CCC	CAT	641
Val	Cys	Ser	Ser	Ser	Leu	Pro	Ala	Arg	Lys	Pro	Pro	Asn	Glu	Pro	His	
	170						175						180			
GTT	TAC	TTG	CCC	GGT	TTA	ATA	ATT	CTG	CTT	CTC	TTC	GCG	TCT	GTG	GCC	689
Val	Tyr	Leu	Pro	Gly	Leu	Ile	Ile	Leu	Leu	Leu	Phe	Ala	Ser	Val	Ala	
	185					190						195				
CTG	GTG	GCT	GCC	ATC	ATC	TTT	GGC	GTT	TGC	TAT	AGG	AAA	AAA	GGG	AAA	737
Leu	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val	Cys	Tyr	Arg	Lys	Lys	Gly	Lys	
	200				205					210				215		
GCA	CTC	ACA	GCT	AAT	TTG	TGG	CAC	TGG	ATC	AAT	GAG	GCT	TGT	GGC	CGC	785
Ala	Leu	Thr	Ala	Asn	Leu	Trp	His	Trp	Ile	Asn	Glu	Ala	Cys	Gly	Arg	
			220					225						230		
CTA	AGT	GGA	GAT	AAG	GAG	TCC	TCA	GGT	GAC	AGT	TGT	GTC	AGT	ACA	CAC	833
Leu	Ser	Gly	Asp	Lys	Glu	Ser	Ser	Gly	Asp	Ser	Cys	Val	Ser	Thr	His	
			235					240					245			
ACG	GCA	AAC	TTT	GGT	CAG	CAG	GGA	GCA	TGT	GAA	GGT	GTC	TTA	CTG	CTG	881
Thr	Ala	Asn	Phe	Gly	Gln	Gln	Gly	Ala	Cys	Glu	Gly	Val	Leu	Leu	Leu	
	250						255					260				
ACT	CTG	GAG	GAG	AAG	ACA	TTT	CCA	GAA	GAT	ATG	TGC	TAC	CCA	GAT	CAA	929
Thr	Leu	Glu	Glu	Lys	Thr	Phe	Pro	Glu	Asp	Met	Cys	Tyr	Pro	Asp	Gln	
	265					270					275					
GGT	GGT	GTC	TGT	CAG	GGC	ACG	TGT	GTA	GGA	GGT	GGT	CCC	TAC	GCA	CAA	977
Gly	Gly	Val	Cys	Gln	Gly	Thr	Cys	Val	Gly	Gly	Gly	Pro	Tyr	Ala	Gln	
	280				285					290				295		
GGC	GAA	GAT	GCC	AGG	ATG	CTC	TCA	TTG	GTC	AGC	AAG	ACC	GAG	ATA	GAG	1025
Gly	Glu	Asp	Ala	Arg	Met	Leu	Ser	Leu	Val	Ser	Lys	Thr	Glu	Ile	Glu	
			300					305					310			
GAA	GAC	AGC	TTC	AGA	CAG	ATG	CCC	ACA	GAA	GAT	GAA	TAC	ATG	GAC	AGG	1073
Glu	Asp	Ser	Phe	Arg	Gln	Met	Pro	Thr	Glu	Asp	Glu	Tyr	Met	Asp	Arg	
			315					320					325			

CCC Pro	TCC Ser	CAG Gln	CCC Pro	ACA Thr	GAC Asp	CAG Gln	TTA Leu	CTG Leu	TTC Phe	CTC Leu	ACT Thr	GAG Glu	CCT Pro	GGA Gly	AGC Ser	1121							
330																335	340						
AAA Lys	TCC Ser	ACA Thr	CCT Pro	CCT Pro	TTC Phe	TCT Ser	GAA Glu	CCC Pro	CTG Leu	GAG Glu	GTG Val	GGG Gly	GAG Glu	AAT Asn	GAC Asp	1169							
345																350	355						
AGT Ser	TTA Leu	AGC Ser	CAG Gln	TGC Cys	TTC Phe	ACG Thr	GGG Gly	ACA Thr	CAG Gln	AGC Ser	ACA Thr	GTG Val	GGT Gly	TCA Ser	GAA Glu	1217							
360																365	370						
AGC Ser	TGC Cys	AAC Asn	TGC Cys	ACT Thr	GAG Glu	CCC Pro	CTG Leu	TGC Cys	AGG Arg	ACT Thr	GAT Asp	TGG Trp	ACT Thr	CCC Pro	ATG Met	1265							
380																385	390						
TCC Ser	TCT Ser	GAA Glu	AAC Asn	TAC Tyr	TTG Leu	CAA Gln	AAA Lys	GAG Glu	GTG Val	GAC Asp	AGT Ser	GGC Gly	CAT His	TGC Cys	CCG Pro	1313							
395																400	405						
CAC His	TGG Trp	GCA Ala	GCC Ala	AGC Ser	CCC Pro	AGC Ser	CCC Pro	AAC Asn	TGG Trp	GCA Ala	GAT Asp	GTC Val	TGC Cys	ACA Thr	GGC Gly	1361							
410																415	420						
TGC Cys	CGG Arg	AAC Asn	CCT Pro	CCT Pro	GGG Gly	GAG Glu	GAC Asp	TGT Cys	GAA Glu	CCC Pro	CTC Leu	GTG Val	GGT Gly	TCC Ser	CCA Pro	1409							
425																430	435						
AAA Lys	CGT Arg	GGA Gly	CCC Pro	TTG Leu	CCC Pro	CAG Gln	TGC Cys	GCC Ala	TAT Tyr	GGC Gly	ATG Met	GGC Gly	CTT Leu	CCC Pro	CCT Pro	1457							
440																445	450						
GAA Glu	GAA Glu	GAA Glu	GCC Ala	AGC Ser	AGG Arg	ACG Thr	GAG Glu	GCC Ala	AGA Arg	GAC Asp	CAG Gln	CCC Pro	GAG Glu	GAT Asp	GGG Gly	1505							
460																465	470						
GCT Ala	GAT Asp	GGG Gly	AGG Gly	CTC Leu	CCA Pro	AGC Ser	TCA Ser	GCG Ala	AGG Arg	GCA Ala	GGT Gly	GCC Ala	GGG Gly	TCT Ser	GGA Gly	1553							
475																480	485						
AGC Ser	TCC Ser	CCT Pro	GGT Gly	GGC Gly	CAG Gln	TCC Ser	CCT Pro	GCA Ala	TCT Ser	GGA Gly	AAT Asn	GTG Val	ACT Thr	GGA Gly	AAC Asn	1601							
490																495	500						
AGT Ser	AAC Asn	TCC Ser	ACG Thr	TTC Phe	ATC Ile	TCC Ser	AGC Ser	GGG Gly	CAG Gln	GTG Val	ATG Met	AAC Asn	TTC Phe	AAG Lys	GGC Gly	1649							
505																510	515						
GAC Asp	ATC Ile	ATC Ile	GTG Val	GTC Val	TAC Tyr	GTC Val	AGC Ser	CAG Gln	ACC Thr	TCG Ser	CAG Gln	GAG Glu	GGC Gly	GCG Ala	GCG Gly	1697							
520																525	530						
GCG Ala	GCT Ala	GCG Ala	GAG Glu	CCC Pro	ATG Met	GGC Gly	CGC Arg	CCG Pro	GTG Val	CAG Gln	GAG Glu	GAG Glu	ACC Thr	CTG Leu	GCG Ala	1745							
540																545	550						
CGC Arg	CGA Arg	GAC Asp	TCC Ser	TTC Phe	GCG Ala	GGG Gly	AAC Asn	GGC Gly	CCG Pro	CGC Arg	TTC Phe	CCG Pro	GAC Asp	CCG Pro	TGC Cys	1793							
555																560	565						
GGC Gly	GGC Pro	CCC Pro	GAG Glu	GGG Gly	CTG Leu	CGG Arg	GAG Glu	CCG Pro	GAG Glu	AAG Lys	GCC Ala	TCG Ser	AGG Arg	CCG Pro	GTG Val	1841							
570																575	580						
CAG Gln	GAG Glu	CAA Gln	GGC Gly	GGG Gly	GCC Ala	AAG Lys	GCT Ala	TGA Ala	GCGCCCCCA	TGGCTGGGAG						1888							
585																590							
CCCCAAGCTC																GGAGCCAGGG	CTCGCGAGGG	CAGCACCAGCA	GCCTCTGCCC	CAGCCCCGGC			1948
CACCCAGGGA																TCGATCGGTA	CAGTCGAGGA	AGACCACCCG	GCATTCTCTG	CCCACCTTGC			2008
CTTCCAGGAA																ATGGCCTTTT	CAGGAAGTGA	ATTGATGAGG	ACTGTCCCCA	TGCCACGGA			2068
TGCTCAGCAG																CCCGCCGCAC	TGGGGCAGAT	GTCTCCCCCTG	CCACTCCTCA	AACTCGCAGC			2128
AGTAATTTGT																GGCACTATGA	CAGCTATTTT	TATGACTATC	CTGTTCTGTG	GGGGGGGGGT			2188
CTATGTTTTC																CCCCCATATT	TGTATTCCTT	TTTATAACTT	TTCTTGATAT	CTTTCCTCCC			2248

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TCTTTTTTAA TGTAAGGTT TTCTCAAAAA TTCTCCTAAA GGTGAGGGTC TCTTTCTTTT 2308
 CTCTTTTCCT TTTTTCCTTC TTTTTCCTGC AACCTGGCTC TGGCCCAGGC TAGAGTGCAG 2368
 TGGTGCGATT ATAGCCCGGT GCAGCCTCTA ACTCCTGGGC TCAAGCAATC CAAGTGATCC 2428
 TCCCACCTCA ACCTTCGGAG TAGCTGGGAT CACAGCTGCA GGCCACGCC AGCTTCCTCC 2488
 CCCCAGACTCC CCCCCCCCAG AGACACGGTC CCACCATGTT ACCCAGCCTG GTCTCAAAC 2548
 CCCCAGCTAA AGCAGTCTCT CAGCCTCGGC CTCCCAAAGT ACTGGGATTA CAGGCGTGAG 2608
 CCCCCACGCT GGCCTGCTTT ACGTATTTTC TTTTGTGCCC CTGCTCACAG TGTTCCTAGAG 2668
 ATGGCTTTCC CAGTGTGTGT TCATTGTAAA CACTTTTGGG AAAGGGCTAA ACATGTGAGG 2728
 CCTGGAGATA GTTGCTAAGT TGCTAGGAAC ATGTGGTGGG ACTTTCATAT TCTGAAAAAT 2788
 GTTCTATATT CTCATTTTTC TAAAGAAAAG AAAAAAGGAA ACCCGATTTA TTTCTCCTGA 2848
 ATCTTTTTAA GTTGTGTGTC TTCCTTAAGC AGAACTAAGC TCAGTATGTG ACCTTACCCG 2908
 CTAGGTGGTT AATTATTCCT TGCTGGCAGA GGCACCTCAGG TACTTGGTAA GCAAATTTCT 2968
 AAAACTCCAA GTTGCTGCAG CTTGGCATTC TTCTTATTCT AGAGGTCTCT CTGGAAAAGA 3028
 TGGAGAAAAT GAACAGGACA TGGGGCTCCT GAAAGAAAAG GGCCCGGGAA GTTCAAGGAA 3088
 GAATAAAGTT GAAATTTTAA AAAAAA 3115

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 591 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu Lys His Tyr Glu
 1 5 10 15
 His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly Lys Tyr Met Ser
 20 25 30
 Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu Pro Cys Gly Pro
 35 40 45
 Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys Cys Leu Leu His
 50 55 60
 Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val Val Ala Gly Asn
 65 70 75 80
 Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly Tyr His Trp Ser
 85 90 95
 Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys Ala Pro Gly Leu
 100 105 110
 Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr Val Cys Lys Pro
 115 120 125
 Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser Thr Asp Lys Cys
 130 135 140
 Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg Val Glu His His
 145 150 155 160
 Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser Leu Pro Ala Arg
 165 170 175
 Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly Leu Ile Ile Leu
 180 185 190

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Leu	Leu	Phe	Ala	Ser	Val	Ala	Leu	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val
	195						200					205			
Cys	Tyr	Arg	Lys	Lys	Gly	Lys	Ala	Leu	Thr	Ala	Asn	Leu	Trp	His	Trp
	210				215						220				
Ile	Asn	Glu	Ala	Cys	Gly	Arg	Leu	Ser	Gly	Asp	Lys	Glu	Ser	Ser	Gly
	225				230					235					240
Asp	Ser	Cys	Val	Ser	Thr	His	Thr	Ala	Asn	Phe	Gly	Gln	Gln	Gly	Ala
			245						250					255	
Cys	Glu	Gly	Val	Leu	Leu	Leu	Thr	Leu	Glu	Glu	Lys	Thr	Phe	Pro	Glu
			260					265						270	
Asp	Met	Cys	Tyr	Pro	Asp	Gln	Gly	Gly	Val	Cys	Gln	Gly	Thr	Cys	Val
	275					280						285			
Gly	Gly	Gly	Pro	Tyr	Ala	Gln	Gly	Glu	Asp	Ala	Arg	Met	Leu	Ser	Leu
	290					295					300				
Val	Ser	Lys	Thr	Glu	Ile	Glu	Glu	Asp	Ser	Phe	Arg	Gln	Met	Pro	Thr
	305				310					315				320	
Glu	Asp	Glu	Tyr	Met	Asp	Arg	Pro	Ser	Gln	Pro	Thr	Asp	Gln	Leu	Leu
			325						330					335	
Phe	Leu	Thr	Glu	Pro	Gly	Ser	Lys	Ser	Thr	Pro	Pro	Phe	Ser	Glu	Pro
			340					345					350		
Leu	Glu	Val	Gly	Glu	Asn	Asp	Ser	Leu	Ser	Gln	Cys	Phe	Thr	Gly	Thr
		355				360						365			
Gln	Ser	Thr	Val	Gly	Ser	Glu	Ser	Cys	Asn	Cys	Thr	Glu	Pro	Leu	Cys
	370					375					380				
Arg	Thr	Asp	Trp	Thr	Pro	Met	Ser	Ser	Glu	Asn	Tyr	Leu	Gln	Lys	Glu
	385				390					395				400	
Val	Asp	Ser	Gly	His	Cys	Pro	His	Trp	Ala	Ala	Ser	Pro	Ser	Pro	Asn
			405						410					415	
Trp	Ala	Asp	Val	Cys	Thr	Gly	Cys	Arg	Asn	Pro	Pro	Gly	Glu	Asp	Cys
		420						425					430		
Glu	Pro	Leu	Val	Gly	Ser	Pro	Lys	Arg	Gly	Pro	Leu	Pro	Gln	Cys	Ala
		435					440					445			
Tyr	Gly	Met	Gly	Leu	Pro	Pro	Glu	Glu	Glu	Ala	Ser	Arg	Thr	Glu	Ala
	450					455					460				
Arg	Asp	Gln	Pro	Glu	Asp	Gly	Ala	Asp	Gly	Arg	Leu	Pro	Ser	Ser	Ala
	465				470				475					480	
Arg	Ala	Gly	Ala	Gly	Ser	Gly	Ser	Ser	Pro	Gly	Gly	Gln	Ser	Pro	Ala
			485						490					495	
Ser	Gly	Asn	Val	Thr	Gly	Asn	Ser	Asn	Ser	Thr	Phe	Ile	Ser	Ser	Gly
		500						505					510		
Gln	Val	Met	Asn	Phe	Lys	Gly	Asp	Ile	Ile	Val	Val	Tyr	Val	Ser	Gln
		515					520					525			
Thr	Ser	Gln	Glu	Gly	Ala	Ala	Ala	Ala	Ala	Glu	Pro	Met	Gly	Arg	Pro
	530					535					540				
Val	Gln	Glu	Glu	Thr	Leu	Ala	Arg	Arg	Asp	Ser	Phe	Ala	Gly	Asn	Gly
	545				550					555				560	
Pro	Arg	Phe	Pro	Asp	Pro	Cys	Gly	Gly	Pro	Glu	Gly	Leu	Arg	Glu	Pro
			565						570					575	
Glu	Lys	Ala	Ser	Arg	Pro	Val	Gln	Glu	Gln	Gly	Gly	Ala	Lys	Ala	
		580					585						590		

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 1391 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
 (A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
 (B) CLONE: 9D-15C

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 39..1391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCGCTGAGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG CGC GCC	53
Met Ala Pro Arg Ala	
1 5	
CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTC TGC GCG CTG CTC	101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Cys Ala Leu Leu	
10 15 20	
GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG	149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu	
25 30 35	
AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA	197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly	
40 45 50	
AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG	245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu	
55 60 65	
CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA	293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys	
70 75 80 85	
TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG	341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val	
GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG	389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly	
TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC	437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys	
GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA	485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr	
GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC	533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser	
ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA	581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg	
GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT	629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser	
775 780 785 185	
190	
CTG CCA GCT AGA AAA CCA CCA AAT GAA CCC CAT GTT TAC TTG CCC GGT	677
Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly	
790 795 800 805200	

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TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC CTG GTG GCT GCC ATC	725
Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile	
810 815 820215	
220	
ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA GCA CTC ACA GCT AAT	773
Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn	
825 830 835 230 235	
TTG TGG CAC TGG ATC AAT GAG GCT TGT GGC CGC CTA AGT GGA GAT AAG	821
Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys	
840 845 850 250	
255	
GAG TCC TCA GGT GAC AGT TGT GTC AGT ACA CAC ACG GCA AAC TTT GGT	869
Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly	
855 860 865 265	
270	
CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG ACT CTG GAG GAG AAG	917
Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys	
870 875 880 885280	
ACA TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA GGT GGT GTC TGT CAG	965
Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys Gln	
890 895 900295	
300	
GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGC GAA GAT GCC AGG	1013
Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg	
905 910 915 310 315	
ATG CTC TCA TTG GTC AGC AAG ACC GAG ATA GAG GAA GAC AGC TTC AGA	1061
Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg	
920 925 930 330	
335	
CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG CCC TCC CAG CCC ACA	1109
Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr	
935 940 945 345	
350	
GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT CCT	1157
Asp Gln Leu Leu Phe Leu Thr Gly Pro Gly Ser Lys Ser Thr Pro Pro	
950 955 960 965360	
TTC TCT GAA CCC CTG GAG GTG GGG GAG AAT GAC AGT TTA AGC CAG TGC	1205
Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys	
970 975 980	
TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA AGC TGC AAC TGC ACT	1253
Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr	
985 990 995	
GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG TCC TCT GAA AAC TAC	1301
Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr	
1000 1005 1010	
TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG CAC TGG GCA GCC AGC	1349
Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser	
1015 1020 1025	
CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC	1391
Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn	
1030 1035 1040 0	

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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 451 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```

Met Ala Pro Arg Ala Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
 1             5             10             15
Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
 20             25             30
Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
 35             40             45
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
 50             55             60
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
 65             70             75             80
Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
 85             90             95
Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
100             105             110
Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
115             120             125
Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
130             135             140
Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
145             150             155             160
Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
165             170             175
Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
180             185             190
Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
195             200             205
Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
210             215             220
Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
225             230             235             240
Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
245             250             255
Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
260             265             270
Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
275             280             285
Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
290             295             300
Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
305             310             315             320
Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
325             330             335
Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
340             345             350
Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser

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355	360	365
Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp		
370	375	380
Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu		
385	390	395 400
Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met		
	405 410	415
Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro		
	420 425	430
His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly		
	435 440	445
Cys Arg Asn		
450		

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3136 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
- (vii) IMMEDIATE SOURCE:
 - (A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
 - (B) CLONE: FULL LENGTH RANK
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 39..1886

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCGCTGAGGC CGCGCGCCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG CGC GCC	53
Met Ala Pro Arg Ala	
1 5	
CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTG CTC TGC GCG CTG CTC	101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu Leu	
10 15 20	
GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG	149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu	
25 30 35	
AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA	197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly	
40 45 50	
AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG	245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu	
55 60 65	
CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA	293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys	
70 75 80 85	
TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG	341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val	
90 95 100	
GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG	389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly	

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105	110	115	
TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys 120 125 130			437
GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr 135 140 145			485
GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser 150 155 160 165			533
ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg 170 175 180			581
GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser 185 190 195			629
CTG CCA GCT AGA AAA CCA CCA AAT GAA CCC CAT GTT TAC TTG CCC GGT Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly 200 205 210			677
TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC CTG GTG GCT GCC ATC Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile 215 220 225			725
ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA GCA CTC ACA GCT AAT Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn 230 235 240 245			773
TTG TGG CAC TGG ATC AAT GAG GCT TGT GGC CGC CTA AGT GGA GAT AAG Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys 250 255 260			821
GAG TCC TCA GGT GAC AGT TGT GTC AGT ACA CAC ACG GCA AAC TTT GGT Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly 265 270 275			869
CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG ACT CTG GAG GAG AAG Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys 280 285 290			917
ACA TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA GGT GGT GTC TGT CAG Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys Gln 295 300 305			965
GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGC GAA GAT GCC AGG Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg 310 315 320 325			1013
ATG CTC TCA TTG GTC AGC AAG ACC GAG ATA GAG GAA GAC AGC TTC AGA Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg 330 335 340			1061
CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG CCC TCC CAG CCC ACA Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr 345 350 355			1109
GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT CCT Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro 360 365 370			1157
TTC TCT GAA CCC CTG GAG GTG GGG GAG AAT GAC AGT TTA AGC CAG TGC Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys 375 380 385			1205
TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA AGC TGC AAC TGC ACT Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr 390 395 400 405			1253
GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG TCC TCT GAA AAC TAC Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr 410 415 420			1301
TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG CAC TGG GCA GCC AGC			1349

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AGTTGCTAGG AACATGTGGT GGGACTTTCA TATTCTGAAA AATGTTCTAT ATTCTCATTT	2826
TTCTAAAAGA AAGAAAAAAG GAAACCCGAT TTATTTCTCC TGAATCTTTT TAAGTTTGTG	2886
TCGTTCCCTTA AGCAGAACTA AGCTCAGTAT GTGACCTTAC CCGCTAGGTG GTTAATTTAT	2946
CCATGCTGGC AGAGGCACTC AGGTACTTGG TAAGCAAATT TCTAAACTC CAAGTTGCTG	3006
CAGCTTGGCA TTCTTCTTAT TCTAGAGGTC TCTCTGGAAA AGATGGAGAA AATGAACAGG	3066
ACATGGGGCT CCTGGAAAGA AAGGGCCCGG GAAGTTCAAG GAAGAATAAA GTTGAAATTT	3126
TAAAAAATAA	3136

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 616 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met	Ala	Pro	Arg	Ala	Arg	Arg	Arg	Arg	Pro	Leu	Phe	Ala	Leu	Leu	Leu	1	5	10	15
Leu	Cys	Ala	Leu	Leu	Ala	Arg	Leu	Gln	Val	Ala	Leu	Gln	Ile	Ala	Pro	20	25	30	
Pro	Cys	Thr	Ser	Glu	Lys	His	Tyr	Glu	His	Leu	Gly	Arg	Cys	Cys	Asn	35	40	45	
Lys	Cys	Glu	Pro	Gly	Lys	Tyr	Met	Ser	Ser	Lys	Cys	Thr	Thr	Thr	Ser	50	55	60	
Asp	Ser	Val	Cys	Leu	Pro	Cys	Gly	Pro	Asp	Glu	Tyr	Leu	Asp	Ser	Trp	65	70	75	80
Asn	Glu	Glu	Asp	Lys	Cys	Leu	Leu	His	Lys	Val	Cys	Asp	Thr	Gly	Lys	85	90	95	
Ala	Leu	Val	Ala	Val	Val	Ala	Gly	Asn	Ser	Thr	Thr	Pro	Arg	Arg	Cys	100	105	110	
Ala	Cys	Thr	Ala	Gly	Tyr	His	Trp	Ser	Gln	Asp	Cys	Glu	Cys	Cys	Arg	115	120	125	
Arg	Asn	Thr	Glu	Cys	Ala	Pro	Gly	Leu	Gly	Ala	Gln	His	Pro	Leu	Gln	130	135	140	
Leu	Asn	Lys	Asp	Thr	Val	Cys	Lys	Pro	Cys	Leu	Ala	Gly	Tyr	Phe	Ser	145	150	155	160
Asp	Ala	Phe	Ser	Ser	Thr	Asp	Lys	Cys	Arg	Pro	Trp	Thr	Asn	Cys	Thr	165	170	175	
Phe	Leu	Gly	Lys	Arg	Val	Glu	His	His	Gly	Thr	Glu	Lys	Ser	Asp	Ala	180	185	190	
Val	Cys	Ser	Ser	Ser	Leu	Pro	Ala	Arg	Lys	Pro	Pro	Asn	Glu	Pro	His	195	200	205	
Val	Tyr	Leu	Pro	Gly	Leu	Ile	Ile	Leu	Leu	Leu	Phe	Ala	Ser	Val	Ala	210	215	220	
Leu	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val	Cys	Tyr	Arg	Lys	Lys	Gly	Lys	225	230	235	240
Ala	Leu	Thr	Ala	Asn	Leu	Trp	His	Trp	Ile	Asn	Glu	Ala	Cys	Gly	Arg	245	250	255	
Leu	Ser	Gly	Asp	Lys	Glu	Ser	Ser	Gly	Asp	Ser	Cys	Val	Ser	Thr	His	260	265	270	
Thr	Ala	Asn	Phe	Gly	Gln	Gln	Gly	Ala	Cys	Glu	Gly	Val	Leu	Leu	Leu	275	280	285	

-continued

Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
 290 295 300
 Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
 305 310 315 320
 Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
 325 330 335
 Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
 340 345 350
 Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
 355 360 365
 Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
 370 375 380
 Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
 385 390 395 400
 Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
 405 410 415
 Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
 420 425 430
 His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
 435 440 445
 Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro
 450 455 460
 Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro
 465 470 475 480
 Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly
 485 490 495
 Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly
 500 505 510
 Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn
 515 520 525
 Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly
 530 535 540
 Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala
 545 550 555 560
 Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala
 565 570 575
 Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys
 580 585 590
 Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val
 595 600 605
 Gln Glu Gln Gly Gly Ala Lys Ala
 610 615

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:

- (B) CLONE: FLAG_ peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

-continued

Asp Tyr Lys Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 232 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Human

(vii) IMMEDIATE SOURCE:

- (B) CLONE: IgG1 Fc mutein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15
Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95
Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110
Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145 150 155 160
His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220
Ser Leu Ser Leu Ser Pro Gly Lys
225 230

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

-continued

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: CMV (R2780 Leader)

(ix) FEATURE:

(D) OTHER INFORMATION: Met1-Arg28 is the actual leader peptide;
Arg29 strengthens the furin cleavage site; nucleotides
encoding eThr30 and Ser31 add a Spel site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```

Met Ala Arg Arg Leu Trp Ile Leu Ser Leu Leu Ala Val Thr Leu Thr
1           5           10           15
Val Ala Leu Ala Ala Pro Ser Gln Lys Ser Lys Arg Arg Thr Ser
          20           25           30

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(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1630 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus musculus

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: <Unknown>

(B) CLONE: RANKL

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 3..884

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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CC GGC GTC CCA CAC GAG GGT CCG CTG CAC CCC GCG CCT TCT GCA CCG      47
Gly Val Pro His Glu Gly Pro Leu His Pro Ala Pro Ser Ala Pro
1           5           10           15

GCT CCG GCG CCG CCA CCC GCC GCC TCC CGC TCC ATG TTC CTG GCC CTC      95
Ala Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser Met Phe Leu Ala Leu
          20           25           30

CTG GGG CTG GGA CTG GGC CAG GTG GTC TGC AGC ATC GCT CTG TTC CTG      143
Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Ile Ala Leu Phe Leu
          35           40           45

TAC TTT CGA GCG CAG ATG GAT CCT AAC AGA ATA TCA GAA GAC AGC ACT      191
Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr
          50           55           60

CAC TGC TTT TAT AGA ATC CTG AGA CTC CAT GAA AAC GCA GAT TTG CAG      239
His Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Gln
          65           70           75

GAC TCG ACT CTG GAG AGT GAA GAC ACA CTA CCT GAC TCC TGC AGG AGG      287
Asp Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg
          80           85           90           95

ATG AAA CAA GCC TTT CAG GGG GCC GTG CAG AAG GAA CTG CAA CAC ATT      335
Met Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile
          100           105           110

GTG GGG CCA CAG CGC TTC TCA GGA GCT CCA GCT ATG ATG GAA GGC TCA      383
Val Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser
          115           120           125

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TGG TTG GAT GTG GCC CAG CGA GGC AAG CCT GAG GCC CAG CCA TTT GCA Trp Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala 130 135 140	431
CAC CTC ACC ATC AAT GCT GCC AGC ATC CCA TCG GGT TCC CAT AAA GTC His Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val 145 150 155	479
ACT CTG TCC TCT TGG TAC CAC GAT CGA GGC TGG GCC AAG ATC TCT AAC Thr Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn 160 165 170 175	527
ATG ACG TTA AGC AAC GGA AAA CTA AGG GTT AAC CAA GAT GGC TTC TAT Met Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr 180 185 190	575
TAC CTG TAC GCC AAC ATT TGC TTT CGG CAT CAT GAA ACA TCG GGA AGC Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser 195 200 205	623
GTA CCT ACA GAC TAT CTT CAG CTG ATG GTG TAT GTC GTT AAA ACC AGC Val Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser 210 215 220	671
ATC AAA ATC CCA AGT TCT CAT AAC CTG ATG AAA GGA GGG AGC ACG AAA Ile Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys 225 230 235	719
AAC TGG TCG GGC AAT TCT GAA TTC CAC TTT TAT TCC ATA AAT GTT GGG Asn Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly 240 245 250 255	767
GGA TTT TTC AAG CTC CGA GCT GGT GAA GAA ATT AGC ATT CAG GTG TCC Gly Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser 260 265 270	815
AAC CCT TCC CTG CTG GAT CCG GAT CAA GAT GCG ACG TAC TTT GGG GCT Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala 275 280 285	863
TTC AAA GTT CAG GAC ATA GAC TGAGACTCAT TTCGTGGAAC ATTAGCATGG Phe Lys Val Gln Asp Ile Asp 290	914
ATGTCCTAGA TGTTTGGA AAA CTTCTTAAAA AATGGATGAT GTCTATACAT GTGTAAGACT	974
ACTAAGAGAC ATGGCCACG GTGTATGAAA CTCACAGCCC TCTCTCTGA GCCTGTACAG	1034
GTGTGTATA TGTAAGTCC ATAGGTGATG TTAGATTCAT GGTGATTACA CAACGGTTTT	1094
ACAATTTTGT AATGATTTCC TAGAATTGAA CCAGATTGGG AGAGGTATTC CGATGCTTAT	1154
GAAAACTTA CACGTGAGCT ATGGAAGGGG GTCACAGTCT CTGGGTCTAA CCCCTGGACA	1214
TGTGCCACTG AGAACCTTGA AATTAAGAGG ATGCCATGTC ATTGCAAAGA AATGATAGTG	1274
TGAAGGGTTA AGTTCTTTTG AATTGTTACA TTGCGCTGGG ACCTGCAAAAT AAGTTCTTTT	1334
TTTCTAATGA GGAGAGAAAA ATATATGTAT TTTTATATAA TGTCTAAAGT TATATTCAG	1394
GTGTAATGTT TTCTGTGCAA AGTTTTGTAA ATTATATTTG TGCTATAGTA TTTGATTCAA	1454
AATATTTAAA AATGCTCTAC TGTGACATA TTTAATGTTT TAAATGTACA GATGTATTTA	1514
ACTGGTGAC TTTGTAATTC CCCTGAAGGT ACTCGTAGCT AAGGGGGCAG AATACTGTTT	1574
CTGGTGACCA CATGTAGTTT ATTTCTTTAT TCTTTTAAAC TTAATAGAGT CTTCAG	1630

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 294 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Gly Val Pro His Glu Gly Pro Leu His Pro Ala Pro Ser Ala Pro Ala
 1 5 10 15
 Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser Met Phe Leu Ala Leu Leu
 20 25 30
 Gly Leu Gly Leu Gly Gln Val Val Cys Ser Ile Ala Leu Phe Leu Tyr
 35 40 45
 Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr His
 50 55 60
 Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Gln Asp
 65 70 75 80
 Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg Met
 85 90 95
 Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile Val
 100 105 110
 Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser Trp
 115 120 125
 Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala His
 130 135 140
 Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val Thr
 145 150 155 160
 Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn Met
 165 170 175
 Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr Tyr
 180 185 190
 Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser Val
 195 200 205
 Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser Ile
 210 215 220
 Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys Asn
 225 230 235 240
 Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly Gly
 245 250 255
 Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser Asn
 260 265 270
 Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe
 275 280 285
 Lys Val Gln Asp Ile Asp
 290

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 954 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: <Unknown>

-continued

(B) CLONE: huRANKL (full length)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..951

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

ATG CGC CGC GCC AGC AGA GAC TAC ACC AAG TAC CTG CGT GGC TCG GAG	48
Met Arg Arg Ala Ser Arg Asp Tyr Thr Lys Tyr Leu Arg Gly Ser Glu	
1 5 10 15	
GAG ATG GGC GGC GGC CCC GGA GCC CCG CAC GAG GGC CCC CTG CAC GCC	96
Glu Met Gly Gly Gly Pro Gly Ala Pro His Glu Gly Pro Leu His Ala	
20 25 30	
CCG CCG CCG CCT GCG CCG CAC CAG CCC CCC GCC GCC TCC CGC TCC ATG	144
Pro Pro Pro Pro Ala Pro His Gln Pro Pro Ala Ala Ser Arg Ser Met	
35 40 45	
TTC GTG GCC CTC CTG GGG CTG GGG CTG GGC CAG GTT GTC TGC AGC GTC	192
Phe Val Ala Leu Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Val	
50 55 60	
GCC CTG TTC TTC TAT TTC AGA GCG CAG ATG GAT CCT AAT AGA ATA TCA	240
Ala Leu Phe Phe Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser	
65 70 75 80	
GAA GAT GGC ACT CAC TGC ATT TAT AGA ATT TTG AGA CTC CAT GAA AAT	288
Glu Asp Gly Thr His Cys Ile Tyr Arg Ile Leu Arg Leu His Glu Asn	
85 90 95	
GCA GAT TTT CAA GAC ACA ACT CTG GAG AGT CAA GAT ACA AAA TTA ATA	336
Ala Asp Phe Gln Asp Thr Thr Leu Glu Ser Gln Asp Thr Lys Leu Ile	
100 105 110	
CCT GAT TCA TGT AGG AGA ATT AAA CAG GCC TTT CAA GGA GCT GTG CAA	384
Pro Asp Ser Cys Arg Arg Ile Lys Gln Ala Phe Gln Gly Ala Val Gln	
115 120 125	
AAG GAA TTA CAA CAT ATC GTT GGA TCA CAG CAC ATC AGA GCA GAG AAA	432
Lys Glu Leu Gln His Ile Val Gly Ser Gln His Ile Arg Ala Glu Lys	
130 135 140	
GCG ATG GTG GAT GGC TCA TGG TTA GAT CTG GCC AAG AGG AGC AAG CTT	480
Ala Met Val Asp Gly Ser Trp Leu Asp Leu Ala Lys Arg Ser Lys Leu	
145 150 155 160	
GAA GCT CAG CCT TTT GCT CAT CTC ACT ATT AAT GCC ACC GAC ATC CCA	528
Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Thr Asp Ile Pro	
165 170 175	
TCT GGT TCC CAT AAA GTG AGT CTG TCC TCT TGG TAC CAT GAT CGG GGT	576
Ser Gly Ser His Lys Val Ser Leu Ser Ser Trp Tyr His Asp Arg Gly	
180 185 190	
TGG GCC AAG ATC TCC AAC ATG ACT TTT AGC AAT GGA AAA CTA ATA GTT	624
Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys Leu Ile Val	
195 200 205	
AAT CAG GAT GGC TTT TAT TAC CTG TAT GCC AAC ATT TGC TTT CGA CAT	672
Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His	
210 215 220	
CAT GAA ACT TCA GGA GAC CTA GCT ACA GAG TAT CTT CAA CTA ATG GTG	720
His Glu Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val	
225 230 235 240	
TAC GTC ACT AAA ACC AGC ATC AAA ATC CCA AGT TCT CAT ACC CTG ATG	768
Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His Thr Leu Met	
245 250 255	
AAA GGA GGA AGC ACC AAG TAT TGG TCA GGG AAT TCT GAA TTC CAT TTT	816
Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu Phe His Phe	
260 265 270	
TAT TCC ATA AAC GTT GGT GGA TTT TTT AAG TTA CGG TCT GGA GAG GAA	864
Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ser Gly Glu Glu	
275 280 285	

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ATC AGC ATC GAG GTC TCC AAC CCC TCC TTA CTG GAT CCG GAT CAG GAT 912
 Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp
 290 295 300

GCA ACA TAC TTT GGG GCT TTT AAA GTT CGA GAT ATA GAT TGA 954
 Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp
 305 310 315

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 317 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Arg Arg Ala Ser Arg Asp Tyr Thr Lys Tyr Leu Arg Gly Ser Glu
 1 5 10 15
 Glu Met Gly Gly Gly Pro Gly Ala Pro His Glu Gly Pro Leu His Ala
 20 25 30
 Pro Pro Pro Pro Ala Pro His Gln Pro Pro Ala Ala Ser Arg Ser Met
 35 40 45
 Phe Val Ala Leu Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Val
 50 55 60
 Ala Leu Phe Phe Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser
 65 70 75 80
 Glu Asp Gly Thr His Cys Ile Tyr Arg Ile Leu Arg Leu His Glu Asn
 85 90 95
 Ala Asp Phe Gln Asp Thr Thr Leu Glu Ser Gln Asp Thr Lys Leu Ile
 100 105 110
 Pro Asp Ser Cys Arg Arg Ile Lys Gln Ala Phe Gln Gly Ala Val Gln
 115 120 125
 Lys Glu Leu Gln His Ile Val Gly Ser Gln His Ile Arg Ala Glu Lys
 130 135 140
 Ala Met Val Asp Gly Ser Trp Leu Asp Leu Ala Lys Arg Ser Lys Leu
 145 150 155 160
 Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Thr Asp Ile Pro
 165 170 175
 Ser Gly Ser His Lys Val Ser Leu Ser Ser Trp Tyr His Asp Arg Gly
 180 185 190
 Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys Leu Ile Val
 195 200 205
 Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His
 210 215 220
 His Glu Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val
 225 230 235 240
 Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His Thr Leu Met
 245 250 255
 Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu Phe His Phe
 260 265 270
 Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ser Gly Glu Glu
 275 280 285
 Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp
 290 295 300
 Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp

-continued

305

310

315

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1878 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Murine

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Murine Fetal Liver Epithelium
- (B) CLONE: muRANK

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

ATG GCC CCG CGC GCC CGG CGG CGC CGC CAG CTG CCC GCG CCG CTG CTG	48
Met Ala Pro Arg Ala Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu	
1 5 10 15	
GCG CTC TGC GTG CTG CTC GTT CCA CTG CAG GTG ACT CTC CAG GTC ACT	96
Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr	
20 25 30	
CCT CCA TGC ACC CAG GAG AGG CAT TAT GAG CAT CTC GGA CGG TGT TGC	144
Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys	
35 40 45	
AGC AGA TGC GAA CCA GGA AAG TAC CTG TCC TCT AAG TGC ACT CCT ACC	192
Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr	
50 55 60	
TCC GAC AGT GTG TGT CTG CCC TGT GGC CCC GAT GAG TAC TTG GAC ACC	240
Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr	
65 70 75 80	
TGG AAT GAA GAA GAT AAA TGC TTG CTG CAT AAA GTC TGT GAT GCA GGC	288
Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly	
85 90 95	
AAG GCC CTG GTG GCG GTG GAT CCT GGC AAC CAC ACG GCC CCG CGT CGC	336
Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg	
100 105 110	
TGT GCT TGC ACG GCT GGC TAC CAC TGG AAC TCA GAC TGC GAG TGC TGC	384
Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys	
115 120 125	
CGC AGG AAC ACG GAG TGT GCA CCT GGC TTC GGA GCT CAG CAT CCC TTG	432
Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu	
130 135 140	
CAG CTC AAC AAG GAT ACG GTG TGC ACA CCC TGC CTC CTG GGC TTC TTC	480
Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe	
145 150 155 160	
TCA GAT GTC TTT TCG TCC ACA GAC AAA TGC AAA CCT TGG ACC AAC TGC	528
Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys	
165 170 175	
ACC CTC CTT GGA AAG CTA GAA GCA CAC CAG GGG ACA ACG GAA TCA GAT	576
Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp	
180 185 190	
GTG GTC TGC AGC TCT TCC ATG ACA CTG AGG AGA CCA CCC AAG GAG GCC	624

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Val	Val	Cys	Ser	Ser	Ser	Met	Thr	Leu	Arg	Arg	Pro	Pro	Lys	Glu	Ala	
195							200					205				
CAG	GCT	TAC	CTG	CCC	AGT	CTC	ATC	GTT	CTG	CTC	CTC	TTC	ATC	TCT	GTG	672
Gln	Ala	Tyr	Leu	Pro	Ser	Leu	Ile	Val	Leu	Leu	Leu	Phe	Ile	Ser	Val	
210						215					220					
GTA	GTA	GTG	GCT	GCC	ATC	ATC	TTC	GGC	GTT	TAC	TAC	AGG	AAG	GGA	GGG	720
Val	Val	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val	Tyr	Tyr	Arg	Lys	Gly	Gly	
225					230					235					240	
AAA	GCG	CTG	ACA	GCT	AAT	TTG	TGG	AAT	TGG	GTC	AAT	GAT	GCT	TGC	AGT	768
Lys	Ala	Leu	Thr	Ala	Asn	Leu	Trp	Asn	Trp	Val	Asn	Asp	Ala	Cys	Ser	
				245					250					255		
AGT	CTA	AGT	GGA	AAT	AAG	GAG	TCC	TCA	GGG	GAC	CGT	TGT	GCT	GGT	TCC	816
Ser	Leu	Ser	Gly	Asn	Lys	Glu	Ser	Ser	Gly	Asp	Arg	Cys	Ala	Gly	Ser	
			260						265					270		
CAC	TCG	GCA	ACC	TCC	AGT	CAG	CAA	GAA	GTG	TGT	GAA	GGT	ATC	TTA	CTA	864
His	Ser	Ala	Thr	Ser	Ser	Gln	Gln	Glu	Val	Cys	Glu	Gly	Ile	Leu	Leu	
		275					280						285			
ATG	ACT	CGG	GAG	GAG	AAG	ATG	GTT	CCA	GAA	GAC	GGT	GCT	GGA	GTC	TGT	912
Met	Thr	Arg	Glu	Glu	Lys	Met	Val	Pro	Glu	Asp	Gly	Ala	Gly	Val	Cys	
	290					295						300				
GGG	CCT	GTG	TGT	GCG	GCA	GGT	GGG	CCC	TGG	GCA	GAA	GTC	AGA	GAT	TCT	960
Gly	Pro	Val	Cys	Ala	Ala	Gly	Gly	Pro	Trp	Ala	Glu	Val	Arg	Asp	Ser	
305					310						315				320	
AGG	ACG	TTC	ACA	CTG	GTC	AGC	GAG	GTT	GAG	ACG	CAA	GGA	GAC	CTC	TCG	1008
Arg	Thr	Phe	Thr	Leu	Val	Ser	Glu	Val	Glu	Thr	Gln	Gly	Asp	Leu	Ser	
				325					330					335		
AGG	AAG	ATT	CCC	ACA	GAG	GAT	GAG	TAC	ACG	GAC	CGG	CCC	TCG	CAG	CCT	1056
Arg	Lys	Ile	Pro	Thr	Glu	Asp	Glu	Tyr	Thr	Asp	Arg	Pro	Ser	Gln	Pro	
			340					345						350		
TCG	ACT	GGT	TCA	CTG	CTC	CTA	ATC	CAG	CAG	GGA	AGC	AAA	TCT	ATA	CCC	1104
Ser	Thr	Gly	Ser	Leu	Leu	Leu	Ile	Gln	Gln	Gly	Ser	Lys	Ser	Ile	Pro	
		355					360						365			
CCA	TTC	CAG	GAG	CCC	CTG	GAA	GTG	GGG	GAG	AAC	GAC	AGT	TTA	AGC	CAG	1152
Pro	Phe	Gln	Glu	Pro	Leu	Glu	Val	Gly	Glu	Asn	Asp	Ser	Leu	Ser	Gln	
		370				375					380					
TGT	TTC	ACC	GGG	ACT	GAA	AGC	ACG	GTG	GAT	TCT	GAG	GGC	TGT	GAC	TTC	1200
Cys	Phe	Thr	Gly	Thr	Glu	Ser	Thr	Val	Asp	Ser	Glu	Gly	Cys	Asp	Phe	
	385				390					395					400	
ACT	GAG	CCT	CCG	AGC	AGA	ACT	GAC	TCT	ATG	CCC	GTG	TCC	CCT	GAA	AAG	1248
Thr	Glu	Pro	Pro	Ser	Arg	Thr	Asp	Ser	Met	Pro	Val	Ser	Pro	Glu	Lys	
				405					410					415		
CAC	CTG	ACA	AAA	GAA	ATA	GAA	GGT	GAC	AGT	TGC	CTC	CCC	TGG	GTG	GTC	1296
His	Leu	Thr	Lys	Glu	Ile	Glu	Gly	Asp	Ser	Cys	Leu	Pro	Trp	Val	Val	
			420					425					430			
AGC	TCC	AAC	TCA	ACA	GAT	GGC	TAC	ACA	GGC	AGT	GGG	AAC	ACT	CCT	GGG	1344
Ser	Ser	Asn	Ser	Thr	Asp	Gly	Tyr	Thr	Gly	Ser	Gly	Asn	Thr	Pro	Gly	
		435					440					445				
GAG	GAC	CAT	GAA	CCC	TTT	CCA	GGG	TCC	CTG	AAA	TGT	GGA	CCA	TTG	CCC	1392
Glu	Asp	His	Glu	Pro	Phe	Pro	Gly	Ser	Leu	Lys	Cys	Gly	Pro	Leu	Pro	
	450					455					460					
CAG	TGT	GCC	TAC	AGC	ATG	GGC	TTT	CCC	AGT	GAA	GCA	GCA	GCC	AGC	ATG	1440
Gln	Cys	Ala	Tyr	Ser	Met	Gly	Phe	Pro	Ser	Glu	Ala	Ala	Ala	Ser	Met	
	465				470					475					480	
GCA	GAG	GCG	GGA	GTA	CGG	CCC	CAG	GAC	AGG	GCT	GAT	GAG	AGG	GGA	GCC	1488
Ala	Glu	Ala	Gly	Val	Arg	Pro	Gln	Asp	Arg	Ala	Asp	Glu	Arg	Gly	Ala	
			485						490					495		
TCA	GGG	TCC	GGG	AGC	TCC	CCC	AGT	GAC	CAG	CCA	CCT	GCC	TCT	GGG	AAC	1536
Ser	Gly	Ser	Gly	Ser	Ser	Pro	Ser	Asp	Gln	Pro	Pro	Ala	Ser	Gly	Asn	
			500					505					510			

-continued

GTG ACT GGA AAC AGT AAC TCC ACG TTC ATC TCT AGC GGG CAG GTG ATG	1584
Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met	
515 520 525	
AAC TTC AAG GGT GAC ATC ATC GTG GTG TAT GTC AGC CAG ACC TCG CAG	1632
Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln	
530 535 540	
GAG GGC CCG GGT TCC GCA GAG CCC GAG TCG GAG CCC GTG GGC CGC CCT	1680
Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro	
545 550 555 560	
GTG CAG GAG GAG ACG CTG GCA CAC AGA GAC TCC TTT GCG GGC ACC GCG	1728
Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala	
565 570 575	
CCG CGC TTC CCC GAC GTC TGT GCC ACC GGG GCT GGG CTG CAG GAG CAG	1776
Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln	
580 585 590	
GGG GCA CCC CGG CAG AAG GAC GGG ACA TCG CGG CCG GTG CAG GAG CAG	1824
Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln	
595 600 605	
GGT GGG GCG CAG ACT TCA CTC CAT ACC CAG GGG TCC GGA CAA TGT GCA	1872
Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala	
610 615 620	
GAA TGA	1878
Glu	
625	

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 625 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Met Ala Pro Arg Ala Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
1 5 10 15

Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
20 25 30

Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
35 40 45

Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
50 55 60

Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
65 70 75 80

Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly
85 90 95

Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
100 105 110

Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
115 120 125

Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu
130 135 140

Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145 150 155 160

Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
165 170 175

Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
180 185 190

-continued

Val	Val	Cys	Ser	Ser	Ser	Met	Thr	Leu	Arg	Arg	Pro	Pro	Lys	Glu	Ala
	195						200					205			
Gln	Ala	Tyr	Leu	Pro	Ser	Leu	Ile	Val	Leu	Leu	Leu	Phe	Ile	Ser	Val
	210					215					220				
Val	Val	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val	Tyr	Tyr	Arg	Lys	Gly	Gly
	225				230					235				240	
Lys	Ala	Leu	Thr	Ala	Asn	Leu	Trp	Asn	Trp	Val	Asn	Asp	Ala	Cys	Ser
			245					250						255	
Ser	Leu	Ser	Gly	Asn	Lys	Glu	Ser	Ser	Gly	Asp	Arg	Cys	Ala	Gly	Ser
		260						265					270		
His	Ser	Ala	Thr	Ser	Ser	Gln	Gln	Glu	Val	Cys	Glu	Gly	Ile	Leu	Leu
		275					280					285			
Met	Thr	Arg	Glu	Glu	Lys	Met	Val	Pro	Glu	Asp	Gly	Ala	Gly	Val	Cys
	290					295					300				
Gly	Pro	Val	Cys	Ala	Ala	Gly	Gly	Pro	Trp	Ala	Glu	Val	Arg	Asp	Ser
	305				310					315				320	
Arg	Thr	Phe	Thr	Leu	Val	Ser	Glu	Val	Glu	Thr	Gln	Gly	Asp	Leu	Ser
			325						330					335	
Arg	Lys	Ile	Pro	Thr	Glu	Asp	Glu	Tyr	Thr	Asp	Arg	Pro	Ser	Gln	Pro
			340					345					350		
Ser	Thr	Gly	Ser	Leu	Leu	Leu	Ile	Gln	Gln	Gly	Ser	Lys	Ser	Ile	Pro
		355					360					365			
Pro	Phe	Gln	Glu	Pro	Leu	Glu	Val	Gly	Glu	Asn	Asp	Ser	Leu	Ser	Gln
	370					375					380				
Cys	Phe	Thr	Gly	Thr	Glu	Ser	Thr	Val	Asp	Ser	Glu	Gly	Cys	Asp	Phe
	385				390					395				400	
Thr	Glu	Pro	Pro	Ser	Arg	Thr	Asp	Ser	Met	Pro	Val	Ser	Pro	Glu	Lys
				405					410					415	
His	Leu	Thr	Lys	Glu	Ile	Glu	Gly	Asp	Ser	Cys	Leu	Pro	Trp	Val	Val
			420					425					430		
Ser	Ser	Asn	Ser	Thr	Asp	Gly	Tyr	Thr	Gly	Ser	Gly	Asn	Thr	Pro	Gly
		435				440						445			
Glu	Asp	His	Glu	Pro	Phe	Pro	Gly	Ser	Leu	Lys	Cys	Gly	Pro	Leu	Pro
	450					455					460				
Gln	Cys	Ala	Tyr	Ser	Met	Gly	Phe	Pro	Ser	Glu	Ala	Ala	Ala	Ser	Met
	465				470					475				480	
Ala	Glu	Ala	Gly	Val	Arg	Pro	Gln	Asp	Arg	Ala	Asp	Glu	Arg	Gly	Ala
			485					490						495	
Ser	Gly	Ser	Gly	Ser	Ser	Pro	Ser	Asp	Gln	Pro	Pro	Ala	Ser	Gly	Asn
			500					505					510		
Val	Thr	Gly	Asn	Ser	Asn	Ser	Thr	Phe	Ile	Ser	Ser	Gly	Gln	Val	Met
		515					520					525			
Asn	Phe	Lys	Gly	Asp	Ile	Ile	Val	Val	Tyr	Val	Ser	Gln	Thr	Ser	Gln
	530				535						540				
Glu	Gly	Pro	Gly	Ser	Ala	Glu	Pro	Glu	Ser	Glu	Pro	Val	Gly	Arg	Pro
	545				550					555				560	
Val	Gln	Glu	Glu	Thr	Leu	Ala	His	Arg	Asp	Ser	Phe	Ala	Gly	Thr	Ala
			565					570					575		
Pro	Arg	Phe	Pro	Asp	Val	Cys	Ala	Thr	Gly	Ala	Gly	Leu	Gln	Glu	Gln
			580					585					590		
Gly	Ala	Pro	Arg	Gln	Lys	Asp	Gly	Thr	Ser	Arg	Pro	Val	Gln	Glu	Gln
	595					600						605			
Gly	Gly	Ala	Gln	Thr	Ser	Leu	His	Thr	Gln	Gly	Ser	Gly	Gln	Cys	Ala

-continued

610	615	620
-----	-----	-----

Glu

625

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15

Gly Ser Thr Gly
 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Asp Tyr Lys Asp Glu
 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

His His His His His His
 5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile
 1 5 10 15

Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
 20 25 30

Arg

What is claimed is:

1. A purified antibody that binds with higher affinity to a human RANKL polypeptide as shown in SEQ ID NO. 13 than to a murine RANKL polypeptide as shown in SEQ ID NO. 11.
2. An antibody according to claim 1 which is a monoclonal antibody.
3. A method of producing a monoclonal antibody according to claim 2, said method comprising culturing a cloned hybridoma cell that produces said antibody.
4. A method of producing a monoclonal antibody according to claim 2, said method comprising injecting into the peritoneal cavity of a rodent a cloned hybridoma cell that produces said antibody.
5. A cloned hybridoma cell that produces a monoclonal antibody according to claim 2.
6. A composition comprising an antibody according to claim 1.
7. A purified antibody that binds with higher affinity to a human RANKL polypeptide than to a murine RANKL polypeptide according to SEQ ID NO. 11, wherein said human RANKL polypeptide is selected from the group consisting of:
 - a) a RANKL polypeptide comprising amino acids 69–313 of SEQ ID NO. 13;
 - b) a RANKL polypeptide comprising amino acids 1–162 of SEQ ID NO. 13;
 - c) a RANKL polypeptide comprising amino acids 162–313 of SEQ ID NO. 13;
 - d) a RANKL polypeptide comprising amino acids 138–317 of SEQ ID NO. 13; and
 - e) a RANKL polypeptide comprising amino acids x to y of SEQ ID NO. 13, wherein x is an amino terminal amino acid between 69 and 162 of SEQ ID NO. 13, and y is a carboxy terminal amino acid between 313 and 317 of SEQ ID NO. 13.
8. An antibody according to claim 7 which is a monoclonal antibody.
9. An antibody according to claim 8, wherein the RANKL polypeptide comprises amino acids 69–313 of SEQ ID NO. 13.
10. An antibody according to claim 8, wherein the RANKL polypeptide comprises amino acids 1–162 of SEQ ID NO. 13.
11. An antibody according to claim 8, wherein the RANKL polypeptide comprises amino acids 162–313 of SEQ ID NO. 13.
12. An antibody according to claim 8, wherein the RANKL polypeptide comprises amino acids 138–317 of SEQ ID NO. 13.
13. A composition comprising an antibody according to claim 8.
14. A purified antibody that binds to a human RANKL polypeptide as shown in SEQ ID NO. 13, but that does not bind to a murine RANKL polypeptide as shown in SEQ ID NO. 11.

15. A purified antibody that binds with higher affinity to a human RANKL polypeptide as shown in SEQ ID NO. 13 than to a murine RANKL polypeptide as shown in SEQ ID NO. 11, wherein said antibody is generated by a method comprising immunizing with a RANKL polypeptide comprising amino acids x to y of SEQ ID NO. 13, wherein x is an amino terminal amino acid between 69 and 162 of SEQ ID NO. 13, and y is a carboxy terminal amino acid between 313 and 317 of SEQ ID NO. 13.
16. A method for generating an antibody, said method comprising immunizing with a RANKL polypeptide selected from the group consisting of:
 - a) a polypeptide comprising amino acids 1–317 of SEQ ID NO. 13;
 - b) a polypeptide comprising amino acids 69–313 of SEQ ID NO. 13;
 - c) a polypeptide comprising amino acids 1–162 of SEQ ID NO. 13;
 - d) a polypeptide comprising amino acids 162–313 of SEQ ID NO. 13;
 - e) a polypeptide comprising amino acids 138–317 of SEQ ID NO. 13; and
 - f) a polypeptide comprising amino acids x to y of SEQ ID NO. 13, wherein x is an amino terminal amino acid between 69 and 162 of SEQ ID NO. 13, and y is a carboxy terminal amino acid between 313 and 317 of SEQ ID NO. 13.
17. A method for preparing an antibody according to claim 16, wherein the antibody is elicited by immunizing with a RANKL polypeptide comprising amino acids 1–317 of SEQ ID NO. 13.
18. A method for preparing an antibody according to claim 16, wherein the antibody is elicited by immunizing with a RANKL polypeptide comprising amino acids 69–313 of SEQ ID NO. 13.
19. A method for preparing an antibody according to claim 16, wherein the antibody is elicited by immunizing with a RANKL polypeptide comprising amino acids 1–162 of SEQ ID NO. 13.
20. A method for preparing an antibody according to claim 16, wherein the antibody is elicited by immunizing with a RANKL polypeptide comprising amino acids 162–313 of SEQ ID NO. 13.
21. A method for preparing an antibody according to claim 16, wherein the antibody is elicited by immunizing with a RANKL polypeptide comprising amino acids 138–317 of SEQ ID NO. 13.
22. A method for preparing an antibody according to claim 16, wherein the antibody is elicited by immunizing with a RANKL polypeptide comprising amino acids x to y of SEQ ID NO. 13, wherein x is an amino terminal amino acid between 69 and 162 of SEQ ID NO. 13, and y is a carboxy terminal amino acid between 313 and 317 of SEQ ID NO. 13.

* * * * *

ATTACHMENT B

In re U.S. Patent No. 6,740,522

Issued: May 25, 2004

To: Dirk M. Anderson

Assignee: Immunex Corporation

**For: ANTIBODIES AGAINST LIGAND FOR RECEPTOR
ACTIVATOR OF NF-KB**

Application for Patent Term Extension

Customer No. 22852



UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
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Customer No 000000

ISTMT

DATE PRINTED
07/23/2010

IMMUNEX CORPORATION
LAW DEPARTMENT
1201 AMGEN COURT WEST
SEATTLE WA 98119

MAINTENANCE FEE STATEMENT

According to the records of the U.S. Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O. Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,740,522	\$900.00	\$0.00	09/20/07	09/865,363	05/25/04	05/25/01	04	NO	2852-E

ATTACHMENT C

In re U.S. Patent No. 6,740,522

Issued: May 25, 2004

To: Dirk M. Anderson

Assignee: Immunex Corporation

**For: ANTIBODIES AGAINST LIGAND FOR RECEPTOR
ACTIVATOR OF NF-KB**

Application for Patent Term Extension

Customer No. 22852

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,740,522 B2
DATED : May 25, 2004
INVENTOR(S) : Dirk M. Anderson

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 6,

Line 34, "a-helical" should read -- α -helical --.

Line 40, "Several studies have indicated..." should start as new paragraph.

Column 10,

Line 1, delete "to be used, for example, in interfering with RANKL signaling, as components of diagnostic or research assays for RANKL or RANKL activity, or in affinity purification of RANKL."

Column 16,

Line 58, "(pBluescriptSK³¹)" should read -- (pBluescriptSK) --.

Column 24,

Line 34, "(1 μ g/ml)" should read -- (1 ng/ml) --, and "(10 g/ml)" should read -- (10 ng/ml) --.

Column 25,

Line 66, "Dendritic cells are referred..." should start as new paragraph.

Column 27,

Line 56, "C57BU6" should read -- C57BL/6 --.

Signed and Sealed this

First Day of March, 2005

A handwritten signature in black ink, appearing to read "Jon W. Dudas", is written over a rectangular area of the document that has been shaded with a fine dot pattern.

JON W. DUDAS

Director of the United States Patent and Trademark Office

ATTACHMENT D

In re U.S. Patent No. 6,740,522

Issued: May 25, 2004

To: Dirk M. Anderson

Assignee: Immunex Corporation

**For: ANTIBODIES AGAINST LIGAND FOR RECEPTOR
ACTIVATOR OF NF-KB**

Application for Patent Term Extension

Customer No. 22852

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Prolia safely and effectively. See full prescribing information for Prolia.

Prolia™ (denosumab)

Injection, for subcutaneous use

Initial US Approval: 2010

INDICATIONS AND USAGE

Prolia is a RANK ligand (RANKL) inhibitor indicated for:

- Treatment of postmenopausal women with osteoporosis at high risk for fracture (1.1)

DOSAGE AND ADMINISTRATION

- Prolia should be administered by a healthcare professional (2.1)
- Administer 60 mg every 6 months as a subcutaneous injection in the upper arm, upper thigh, or abdomen (2.1)
- Instruct patients to take calcium 1000 mg daily and at least 400 IU vitamin D daily (2.1)

DOSAGE FORMS AND STRENGTHS

- Single-use prefilled syringe containing 60 mg in a 1 mL solution (3)
- Single-use vial containing 60 mg in a 1 mL solution (3)

CONTRAINDICATIONS

- Hypocalcemia (4.1, 5.1)

WARNINGS AND PRECAUTIONS

- Hypocalcemia: Must be corrected before initiating Prolia. May worsen especially in patients with renal impairment. Adequately supplement patients with calcium and vitamin D (5.1)
- Serious infections including skin infections: May occur, including those leading to hospitalization. Advise patients to seek prompt medical

attention if they develop signs or symptoms of infection, including cellulitis (5.2)

- Dermatologic reactions: Dermatitis, rashes, and eczema have been reported. Consider discontinuing Prolia if severe symptoms develop (5.3)
- Osteonecrosis of the jaw: Has been reported with Prolia. Monitor for symptoms (5.4)
- Suppression of bone turnover: Significant suppression has been demonstrated. Monitor for consequences of bone oversuppression (5.5)

ADVERSE REACTIONS

- Most common adverse reactions (> 5% and more common than placebo): back pain, pain in extremity, hypercholesterolemia, musculoskeletal pain, and cystitis. Pancreatitis has been reported in clinical trials (6.1).

To report SUSPECTED ADVERSE REACTIONS, contact Amgen Inc. at 1-800-77-AMGEN (1-800-772-6436) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

USE IN SPECIFIC POPULATIONS

- Pregnancy: Based on animal data, may cause fetal harm. Pregnancy Surveillance Program available (8.1)
- Nursing mothers: May impair mammary gland development and lactation. Discontinue drug or nursing (8.3)
- Pediatric patients: Safety and efficacy not established (8.4)
- Renal impairment: No dose adjustment is necessary in patients with renal impairment. Patients with creatinine clearance < 30 mL/min or receiving dialysis are at risk for hypocalcemia. Supplement with calcium and vitamin D and consider monitoring serum calcium (8.6)

See 17 for PATIENT COUNSELING INFORMATION and Medication Guide.

Revised: 06/2010

FULL PRESCRIBING INFORMATION: CONTENTS*

1 INDICATIONS AND USAGE

- 1.1 Treatment of Postmenopausal Women with Osteoporosis at High Risk for Fracture

2 DOSAGE AND ADMINISTRATION

- 2.1 Recommended Dosage
- 2.2 Preparation and Administration

3 DOSAGE FORMS AND STRENGTHS

4 CONTRAINDICATIONS

- 4.1 Hypocalcemia

5 WARNINGS AND PRECAUTIONS

- 5.1 Hypocalcemia and Mineral Metabolism
- 5.2 Serious Infections
- 5.3 Dermatologic Adverse Reactions
- 5.4 Osteonecrosis of the Jaw
- 5.5 Suppression of Bone Turnover

6 ADVERSE REACTIONS

- 6.1 Clinical Trials Experience

7 DRUG INTERACTIONS

8 USE IN SPECIFIC POPULATIONS

- 8.1 Pregnancy
- 8.3 Nursing Mothers
- 8.4 Pediatric Use
- 8.5 Geriatric Use
- 8.6 Renal Impairment
- 8.7 Hepatic Impairment

10 OVERDOSAGE

11 DESCRIPTION

12 CLINICAL PHARMACOLOGY

- 12.1 Mechanism of Action
- 12.2 Pharmacodynamics
- 12.3 Pharmacokinetics

13 NONCLINICAL TOXICOLOGY

- 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
- 13.2 Animal Toxicology and/or Pharmacology

14 CLINICAL STUDIES

- 14.1 Postmenopausal Women with Osteoporosis

16 HOW SUPPLIED/STORAGE AND HANDLING

17 PATIENT COUNSELING INFORMATION

- 17.1 Hypocalcemia
- 17.2 Serious Infections
- 17.3 Dermatologic Reactions
- 17.4 Osteonecrosis of the Jaw
- 17.5 Schedule of Administration

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Treatment of Postmenopausal Women with Osteoporosis at High Risk for Fracture

Prolia is indicated for the treatment of postmenopausal women with osteoporosis at high risk for fracture, defined as a history of osteoporotic fracture, or multiple risk factors for fracture; or patients who have failed or are intolerant to other available osteoporosis therapy. In postmenopausal women with osteoporosis, Prolia reduces the incidence of vertebral, nonvertebral, and hip fractures [see *Clinical Studies (14.1)*].

2 DOSAGE AND ADMINISTRATION

2.1 Recommended Dosage

Prolia should be administered by a healthcare professional.

The recommended dose of Prolia is 60 mg administered as a single subcutaneous injection once every 6 months. Administer Prolia via subcutaneous injection in the upper arm, the upper thigh, or the abdomen. All patients should receive calcium 1000 mg daily and at least 400 IU vitamin D daily [see *Warnings and Precautions (5.1)*].

If a dose of Prolia is missed, administer the injection as soon as the patient is available. Thereafter, schedule injections every 6 months from the date of the last injection.

2.2 Preparation and Administration

Visually inspect Prolia for particulate matter and discoloration prior to administration whenever solution and container permit. Prolia is a clear, colorless to pale yellow solution that may contain trace amounts of translucent to white proteinaceous particles. Do not use if the solution is discolored or cloudy or if the solution contains many particles or foreign particulate matter.

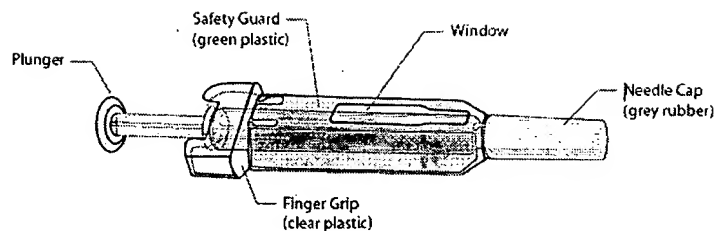
Latex Allergy: People sensitive to latex should not handle the grey needle cap on the single-use prefilled syringe, which contains dry natural rubber (a derivative of latex).

Prior to administration, Prolia may be removed from the refrigerator and brought to room temperature (up to 25°C/77°F) by standing in the original container. This generally takes 15 to 30 minutes. Do not warm Prolia in any other way [see *How Supplied/Storage and Handling (16)*].

Instructions for Prefilled Syringe with Needle Safety Guard

IMPORTANT: In order to minimize accidental needlesticks, the Prolia single-use prefilled syringe will have a green safety guard; manually activate the safety guard after the injection is given.

DO NOT slide the green safety guard forward over the needle before administering the injection; it will lock in place and prevent injection.

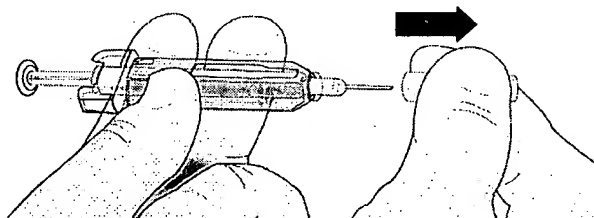


Activate the green safety guard (slide over the needle) after the injection.

The grey needle cap on the single use prefilled syringe contains dry natural rubber (a derivative of latex); people sensitive to latex should not handle the cap.

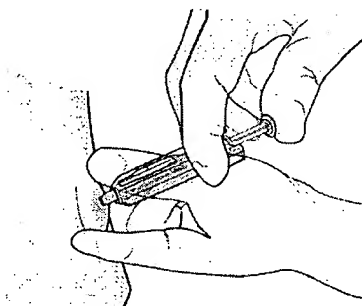
Step 1: Remove Grey Needle Cap

Remove needle cap.



Step 2: Administer Injection

Insert needle and inject all the liquid.



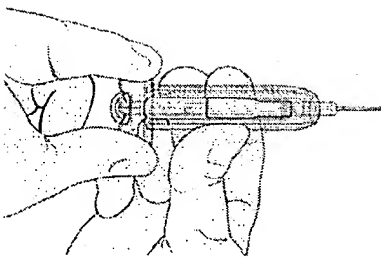
DO NOT put grey needle cap back on needle.

Step 3: Immediately Slide Green Safety Guard Over Needle

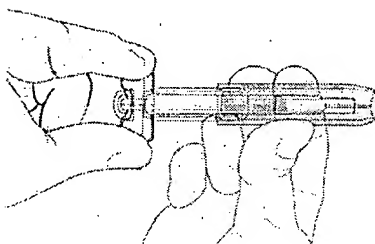
With the *needle pointing away from you*...

Hold the prefilled syringe by the clear plastic finger grip with one hand. Then, with the other hand, grasp the green safety guard by its base and gently slide it towards the needle until the green safety guard locks securely in place and/or you hear a “click.” **DO NOT** grip the green safety guard too firmly – it will move easily if you hold and slide it gently.

Hold clear finger grip.



Gently slide green safety guard over needle and lock securely in place. Do not grip green safety guard too firmly when sliding over needle.



Immediately dispose of the syringe and needle cap in the nearest sharps container. **DO NOT** put the needle cap back on the used syringe.

Instructions for Single-use Vial

For administration of Prolia from the single-use vial, use a 27-gauge needle to withdraw and inject the 1 mL dose. Do not re-enter the vial. Discard vial and any liquid remaining in the vial.

3 DOSAGE FORMS AND STRENGTHS

- 1 mL of a 60 mg/mL solution in a single-use prefilled syringe
- 1 mL of a 60 mg/mL solution in a single-use vial

4 CONTRAINDICATIONS

4.1 Hypocalcemia

Pre-existing hypocalcemia must be corrected prior to initiating therapy with Prolia [see *Warnings and Precautions* (5.1)].

5 WARNINGS AND PRECAUTIONS

5.1 Hypocalcemia and Mineral Metabolism

Hypocalcemia may be exacerbated by the use of Prolia. Pre-existing hypocalcemia must be corrected prior to initiating therapy with Prolia. In patients predisposed to hypocalcemia and disturbances of mineral metabolism (e.g. history of hypoparathyroidism, thyroid surgery, parathyroid surgery, malabsorption syndromes, excision of small intestine, severe renal impairment [creatinine clearance < 30 mL/min] or receiving dialysis), clinical monitoring of calcium and mineral levels (phosphorus and magnesium) is highly recommended.

Hypocalcemia following Prolia administration is a significant risk in patients with severe renal impairment [creatinine clearance < 30 mL/min], or receiving dialysis. Instruct all patients with severe renal impairment, including those receiving dialysis, about the symptoms of hypocalcemia and the importance of maintaining calcium levels with adequate calcium and vitamin D supplementation.

Adequately supplement all patients with calcium and vitamin D [see *Dosage and Administration* (2.1), *Contraindications* (4.1), *Adverse Reactions* (6.1), and *Patient Counseling Information* (17.1)].

5.2 Serious Infections

In a clinical trial of over 7800 women with postmenopausal osteoporosis, serious infections leading to hospitalization were reported more frequently in the Prolia group than in the placebo group [see *Adverse Reactions* (6.1)]. Serious skin infections, as well as infections of the abdomen, urinary tract, and ear, were more frequent in patients treated with Prolia. Endocarditis was also reported more frequently in Prolia-treated subjects. The incidence of opportunistic infections was balanced between placebo and Prolia groups, and the overall incidence of infections was similar between the treatment groups. Advise patients to seek prompt medical attention if they develop signs or symptoms of severe infection, including cellulitis.

Patients on concomitant immunosuppressant agents or with impaired immune systems may be at increased risk for serious infections. Consider the benefit-risk profile in such patients before treating with Prolia. In patients who develop serious infections while on Prolia, prescribers should assess the need for continued Prolia therapy.

5.3 Dermatologic Adverse Reactions

In a large clinical trial of over 7800 women with postmenopausal osteoporosis, epidermal and dermal adverse events such as dermatitis, eczema, and rashes occurred at a significantly higher rate in the Prolia group compared to the placebo group. Most of these events were not specific to the injection site [see *Adverse Reactions* (6.1)]. Consider discontinuing Prolia if severe symptoms develop.

5.4 Osteonecrosis of the Jaw

Osteonecrosis of the jaw (ONJ), which can occur spontaneously, is generally associated with tooth extraction and/or local infection with delayed healing. ONJ has been reported in patients receiving denosumab [see *Adverse Reactions* (6.1)]. A routine oral exam should be performed by the prescriber prior to initiation of Prolia treatment. A dental examination with appropriate preventive dentistry should be considered prior to treatment with Prolia in patients with risk factors for ONJ such as invasive dental procedures (e.g., tooth extraction, dental implants, oral surgery), diagnosis of cancer, concomitant therapies (e.g., chemotherapy, corticosteroids), poor oral hygiene, and co-morbid disorders (e.g., periodontal and/or other pre-existing dental disease, anemia, coagulopathy, infection, ill-fitting dentures). Good oral hygiene practices should be maintained during treatment with Prolia.

For patients requiring invasive dental procedures, clinical judgment of the treating physician and/or oral surgeon should guide the management plan of each patient based on individual benefit-risk assessment.

Patients who are suspected of having or who develop ONJ while on Prolia should receive care by a dentist or an oral surgeon. In these patients, extensive dental surgery to treat ONJ may exacerbate the condition. Discontinuation of Prolia therapy should be considered based on individual benefit-risk assessment.

5.5 Suppression of Bone Turnover

In clinical trials in women with postmenopausal osteoporosis, treatment with Prolia resulted in significant suppression of bone remodeling as evidenced by markers of bone turnover and bone histomorphometry [see *Clinical Pharmacology* (12.2), *Clinical Studies* (14.1)]. The significance of these findings and the effect of long-term treatment with Prolia are unknown. The long-term consequences of the degree of suppression of bone remodeling observed with Prolia may contribute to adverse outcomes such as osteonecrosis of the jaw, atypical fractures, and delayed fracture healing. Monitor patients for these consequences.

6 ADVERSE REACTIONS

The following serious adverse reactions are discussed below and also elsewhere in the labeling:

- Hypocalcemia [see *Warnings and Precautions* (5.1)]
- Serious Infections [see *Warnings and Precautions* (5.2)]
- Dermatologic Adverse Reactions [see *Warnings and Precautions* (5.3)]
- Osteonecrosis of the Jaw [see *Warnings and Precautions* (5.4)]

The most common adverse reactions reported with Prolia are back pain, pain in extremity, musculoskeletal pain, hypercholesterolemia, and cystitis.

The most common adverse reactions leading to discontinuation of Prolia are breast cancer, back pain, and constipation.

The Prolia Postmarketing Active Safety Surveillance Program is available to collect information from prescribers on specific adverse events. Please see www.proliasafety.com or call 1-800-772-6436 for more information about this program.

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of a drug cannot be directly compared to rates in the clinical studies of another drug and may not reflect the rates observed in clinical practice.

Treatment of postmenopausal women with osteoporosis

The safety of Prolia in the treatment of postmenopausal osteoporosis was assessed in a 3-year, randomized, double-blind, placebo-controlled, multinational study of 7808 postmenopausal women aged 60 to 91 years. A total of 3876 women were exposed to placebo and 3886 women were exposed to Prolia administered subcutaneously once every 6 months as a single 60 mg dose. All women were instructed to take at least 1000 mg of calcium and 400 IU of vitamin D supplementation per day.

The incidence of all-cause mortality was 2.3% (n = 90) in the placebo group and 1.8% (n = 70) in the Prolia group. The incidence of nonfatal serious adverse events was 24.2% in the placebo group and 25.0% in the Prolia group. The percentage of patients who withdrew from the study due to adverse events was 2.1% and 2.4% for the placebo and Prolia groups, respectively.

Adverse reactions reported in $\geq 2\%$ of postmenopausal women with osteoporosis and more frequently in the Prolia-treated women than in the placebo-treated women are shown in the table below.

Table 1. Adverse Reactions Occurring in $\geq 2\%$ of Patients with Osteoporosis and More Frequently than in Placebo-treated Patients

SYSTEM ORGAN CLASS Preferred Term	Prolia (N = 3886) n (%)	Placebo (N = 3876) n (%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
Anemia	129 (3.3)	107 (2.8)
CARDIAC DISORDERS		
Angina pectoris	101 (2.6)	87 (2.2)
Atrial fibrillation	79 (2.0)	77 (2.0)
EAR AND LABYRINTH DISORDERS		
Vertigo	195 (5.0)	187 (4.8)
GASTROINTESTINAL DISORDERS		
Abdominal pain upper	129 (3.3)	111 (2.9)
Flatulence	84 (2.2)	53 (1.4)
Gastroesophageal reflux disease	80 (2.1)	66 (1.7)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Edema peripheral	189 (4.9)	155 (4.0)
Asthenia	90 (2.3)	73 (1.9)
INFECTIONS AND INFESTATIONS		
Cystitis	228 (5.9)	225 (5.8)
Upper respiratory tract infection	190 (4.9)	167 (4.3)
Pneumonia	152 (3.9)	150 (3.9)
Pharyngitis	91 (2.3)	78 (2.0)
Herpes zoster	79 (2.0)	72 (1.9)
METABOLISM AND NUTRITION DISORDERS		
Hypercholesterolemia	280 (7.2)	236 (6.1)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
Back pain	1347 (34.7)	1340 (34.6)
Pain in extremity	453 (11.7)	430 (11.1)
Musculoskeletal pain	297 (7.6)	291 (7.5)
Bone pain	142 (3.7)	117 (3.0)
Myalgia	114 (2.9)	94 (2.4)
Spinal osteoarthritis	82 (2.1)	64 (1.7)

SYSTEM ORGAN CLASS Preferred Term	Prolia (N = 3886) n (%)	Placebo (N = 3876) n (%)
NERVOUS SYSTEM DISORDERS		
Sciatica	178 (4.6)	149 (3.8)
PSYCHIATRIC DISORDERS		
Insomnia	126 (3.2)	122 (3.1)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
Rash	96 (2.5)	79 (2.0)
Pruritus	87 (2.2)	82 (2.1)

Hypocalcemia

Decreases in serum calcium levels to less than 8.5 mg/dL were reported in 0.4% women in the placebo group and 1.7% women in the Prolia group at the month 1 visit. The nadir in serum calcium level occurs at approximately day 10 after Prolia dosing in subjects with normal renal function.

In clinical studies, subjects with impaired renal function were more likely to have greater reductions in serum calcium levels compared to subjects with normal renal function. In a study of 55 patients with varying degrees of renal function, serum calcium levels < 7.5 mg/dL or symptomatic hypocalcemia were observed in 5 subjects. These included no subjects in the normal renal function group, 10% of subjects in the CrCL 50 to 80 mL/min group, 29% of subjects in the CrCL < 30 mL/min group, and 29% of subjects in the hemodialysis group. These subjects did not receive calcium and vitamin D supplementation. In a study of 4,550 postmenopausal women with osteoporosis, the mean change from baseline in serum calcium level 10 days after Prolia dosing was -5.5% in subjects with creatinine clearance < 30 mL/min vs. -3.1% in subjects with CrCL ≥ 30 mL/min.

Serious Infections

Receptor activator of nuclear factor kappa-B ligand (RANKL) is expressed on activated T and B lymphocytes and in lymph nodes. Therefore, a RANKL inhibitor such as Prolia may increase the risk of infection.

In the clinical study of 7808 postmenopausal women with osteoporosis, the incidence of infections resulting in death was 0.2% in both placebo and Prolia treatment groups. However, the incidence of nonfatal serious infections was 3.3% in the placebo group and 4.0% in the Prolia group. Hospitalizations due to serious infections in the abdomen (0.7% placebo vs. 0.9% Prolia), urinary tract (0.5% placebo vs. 0.7% Prolia), and ear (0.0% placebo vs. 0.1% Prolia) were reported. Endocarditis was reported in no placebo patients and 3 patients receiving Prolia.

Skin infections, including erysipelas and cellulitis, leading to hospitalization were reported more frequently in patients treated with Prolia (< 0.1% placebo vs. 0.4% Prolia).

There was no imbalance in the reporting of opportunistic infections.

Dermatologic Reactions

A significantly higher number of patients treated with Prolia developed epidermal and dermal adverse events (such as dermatitis, eczema, and rashes), with these events reported in 8.2% of placebo and 10.8%

of Prolia group ($p < 0.0001$). Most of these events were not specific to the injection site [see *Warnings and Precautions* (5.3)].

Osteonecrosis of the Jaw

ONJ has been reported in the osteoporosis clinical trial program in patients treated with Prolia [see *Warnings and Precautions* (5.4)].

Pancreatitis

Pancreatitis was reported in 4 patients (0.1%) in the placebo and 8 patients (0.2%) in the Prolia groups. Of these reports, one subject in the placebo group and all 8 subjects in the Prolia group had serious events including one death in the Prolia group. Several patients had a prior history of pancreatitis. The time from product administration to event occurrence was variable.

New Malignancies

The overall incidence of new malignancies was 4.3% in the placebo and 4.8% in the Prolia groups. New malignancies related to breast (0.7% placebo vs. 0.9% Prolia), reproductive (0.2% placebo vs. 0.5% Prolia), and gastrointestinal systems (0.6% placebo vs. 0.9% Prolia) were reported. A causal relationship to drug exposure has not been established.

Immunogenicity

Denosumab is a human monoclonal antibody. As with all therapeutic proteins, there is potential for immunogenicity. Using an electrochemiluminescent bridging immunoassay, less than 1% (55 out of 8113) of patients treated with Prolia for up to 5 years tested positive for binding antibodies (including pre-existing, transient, and developing antibodies). None of the patients tested positive for neutralizing antibodies, as was assessed using a chemiluminescent cell-based in vitro biological assay. No evidence of altered pharmacokinetic profile, toxicity profile, or clinical response was associated with binding antibody development.

The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of a positive antibody (including neutralizing antibody) test result may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of antibodies to denosumab with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

No drug-drug interaction studies have been conducted with Prolia.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of Prolia in pregnant women. In genetically engineered mice in which RANK ligand (RANKL) was turned off by gene removal (a “knockout mouse”), absence of RANKL (the target of denosumab) caused fetal lymph node agenesis and led to postnatal impairment of dentition and bone growth. Pregnant RANKL knockout mice also showed altered maturation of the maternal mammary gland, leading to impaired lactation postpartum [see *Use in Specific Populations* (8.3)].

Prolia is approved only for use in postmenopausal women. Prolia should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women who become pregnant during Prolia treatment are encouraged to enroll in Amgen's Pregnancy Surveillance Program. Patients or their physicians should call 1-800-77-AMGEN (1-800-772-6436) to enroll.

In an embryofetal developmental study, cynomolgus monkeys received subcutaneous denosumab weekly during organogenesis at doses up to 13-fold higher than the recommended human dose of 60 mg administered once every 6 months based on body weight (mg/kg). No evidence of maternal toxicity or fetal harm was observed. However, this study only assessed fetal toxicity during a period equivalent to the first trimester and fetal lymph nodes were not examined. Monoclonal antibodies are transported across the placenta in a linear fashion as pregnancy progresses, with the largest amount transferred during the third trimester. Potential adverse developmental effects resulting from exposures during the second and third trimesters have not been assessed in animals [see *Nonclinical Toxicology* (13.2)].

8.3 Nursing Mothers

It is not known whether Prolia is excreted into human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Prolia, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

Maternal exposure to Prolia during pregnancy may impair mammary gland development and lactation based on animal studies in pregnant mice lacking the RANK/RANKL signaling pathway that have shown altered maturation of the maternal mammary gland, leading to impaired lactation postpartum [see *Nonclinical Toxicology* (13.2)].

8.4 Pediatric Use

Prolia is not recommended in pediatric patients. The safety and effectiveness of Prolia in pediatric patients have not been established.

Treatment with Prolia may impair bone growth in children with open growth plates and may inhibit eruption of dentition. In neonatal rats, inhibition of RANKL (the target of Prolia therapy) with a construct of osteoprotegerin bound to Fc (OPG-Fc) at doses ≤ 10 mg/kg was associated with inhibition of bone growth and tooth eruption. Adolescent primates dosed with denosumab at 10 and 50 times (10 and 50 mg/kg dose) higher than the recommended human dose of 60 mg administered once every 6 months, based on body weight (mg/kg), had abnormal growth plates [see *Nonclinical Toxicology* (13.2)].

8.5 Geriatric Use

Of the total number of patients in clinical studies of Prolia, 9943 patients (76%) were ≥ 65 years old, while 3576 (27%) were ≥ 75 years old. No overall differences in safety or efficacy were observed between these patients and younger patients and other reported clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

8.6 Renal Impairment

No dose adjustment is necessary in patients with renal impairment.

In clinical studies, patients with severe renal impairment (creatinine clearance < 30 mL/min) or receiving dialysis were at greater risk of developing hypocalcemia. Consider the benefit-risk profile when administering Prolia to patients with severe renal impairment or receiving dialysis. Clinical monitoring of calcium and mineral levels (phosphorus and magnesium) is highly recommended. Adequate intake of calcium and vitamin D is important in patients with severe renal impairment or receiving dialysis [see *Warnings and Precautions* (5.1), *Adverse Reactions* (6.1), and *Clinical Pharmacology* (12.3)].

8.7 Hepatic Impairment

No clinical studies have been conducted to evaluate the effect of hepatic impairment on the pharmacokinetics of Prolia.

10 OVERDOSAGE

There is no experience with overdosage with Prolia.

11 DESCRIPTION

Prolia (denosumab) is a human IgG2 monoclonal antibody with affinity and specificity for human RANKL (receptor activator of nuclear factor kappa-B ligand). Denosumab has an approximate molecular weight of 147 kDa and is produced in genetically engineered mammalian (Chinese hamster ovary) cells.

Prolia is a sterile, preservative-free, clear, colorless to pale yellow solution.

Each 1 mL single-use prefilled syringe of Prolia contains 60 mg denosumab (60mg/mL solution), 4.7% sorbitol, 17 mM acetate, 0.01% polysorbate 20, Water for Injection (USP), and sodium hydroxide to a pH of 5.2.

Each 1 mL single-use vial of Prolia contains 60 mg denosumab (60 mg/mL solution), 4.7% sorbitol, 17 mM acetate, Water for Injection (USP), and sodium hydroxide to a pH of 5.2.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Prolia binds to RANKL, a transmembrane or soluble protein essential for the formation, function, and survival of osteoclasts, the cells responsible for bone resorption. Prolia prevents RANKL from activating its receptor, RANK, on the surface of osteoclasts and their precursors. Prevention of the RANKL/RANK interaction inhibits osteoclast formation, function, and survival, thereby decreasing bone resorption and increasing bone mass and strength in both cortical and trabecular bone.

12.2 Pharmacodynamics

In clinical studies, treatment with 60 mg of Prolia resulted in reduction in the bone resorption marker serum type 1 C-telopeptide (CTX) by approximately 85% by 3 days, with maximal reductions occurring by 1 month. CTX levels were below the limit of assay quantitation (0.049 ng/mL) in 39-68% of subjects 1-3 months after dosing of Prolia. At the end of each dosing interval, CTX reductions were partially attenuated from a maximal reduction of $\geq 87\%$ to $\geq 45\%$ (range: 45% to 80%), as serum denosumab levels diminished, reflecting the reversibility of the effects of Prolia on bone remodeling. These effects were sustained with continued treatment. Upon reinitiation, the degree of inhibition of CTX by Prolia was similar to that observed in patients initiating Prolia treatment.

Consistent with the physiological coupling of bone formation and resorption in skeletal remodeling, subsequent reductions in bone formation markers (i.e., osteocalcin and procollagen type 1 N-terminal peptide [PINP]) were observed starting 1 month after the first dose of Prolia. After discontinuation of Prolia therapy, markers of bone resorption increased to levels 40-60% above pretreatment values but returned to baseline levels within 12 months.

12.3 Pharmacokinetics

In a study conducted in healthy male and female volunteers (n = 73, age range: 18 to 64 years) following a single subcutaneously administered Prolia dose of 60 mg after fasting (at least for 12 hours), the mean maximum denosumab concentration (C_{max}) was 6.75 mcg/mL (standard deviation [SD] = 1.89 mcg/mL). The median time to maximum denosumab concentration (T_{max}) was 10 days (range: 3 to 21 days). After C_{max} , serum denosumab concentrations declined over a period of 4 to 5 months with a mean half-life of 25.4 days (SD = 8.5 days; n = 46). The mean area-under-the-concentration-time curve up to 16 weeks ($AUC_{0-16 \text{ weeks}}$) of denosumab was 316 mcg·day/mL (SD = 101 mcg·day/mL).

No accumulation or change in denosumab pharmacokinetics with time was observed upon multiple dosing of 60 mg subcutaneously administered once every 6 months.

Prolia pharmacokinetics were not affected by the formation of binding antibodies.

A population pharmacokinetic analysis was performed to evaluate the effects of demographic characteristics. This analysis showed no notable differences in pharmacokinetics with age (in postmenopausal women), race, or body weight (36 to 140 kg).

Drug Interactions

No drug-drug interaction studies have been conducted with Prolia.

Specific Populations

Gender: Mean serum denosumab concentration-time profiles observed in a study conducted in healthy men ≥ 50 years were similar to those observed in a study conducted in postmenopausal women using the same dose regimen.

Age: The pharmacokinetics of denosumab was not affected by age across all populations studied whose ages ranged from 28-87 years.

Race: The pharmacokinetics of denosumab was not affected by race.

Renal Impairment: In a study of 55 patients with varying degrees of renal function, including patients on dialysis, the degree of renal impairment had no effect on the pharmacokinetics of denosumab; thus, dose adjustment for renal impairment is not necessary.

Hepatic Impairment: No clinical studies have been conducted to evaluate the effect of hepatic impairment on the pharmacokinetics of denosumab.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity

The carcinogenic potential of denosumab has not been evaluated in long-term animal studies.

Mutagenicity

The genotoxic potential of denosumab has not been evaluated.

Impairment of Fertility

Denosumab had no effect on female fertility or male reproductive organs in monkeys at doses that were 13- to 50-fold higher than the recommended human dose of 60 mg administered once every 6 months, based on body weight (mg/kg).

13.2 Animal Toxicology and/or Pharmacology

Prolia is an inhibitor of osteoclastic bone resorption via inhibition of RANKL.

In ovariectomized monkeys, once-monthly treatment with denosumab suppressed bone turnover and increased bone mineral density (BMD) and strength of cancellous and cortical bone at doses 50-fold higher than the recommended human dose of 60 mg administered once every 6 months, based on body weight (mg/kg). Bone tissue was normal with no evidence of mineralization defects, accumulation of osteoid, or woven bone.

Adolescent primates treated with denosumab at doses > 10 times (10 and 50 mg/kg dose) higher than the recommended human dose of 60 mg administered once every 6 months, based on mg/kg, had abnormal growth plates, considered to be consistent with the pharmacological activity of denosumab [see *Use in Specific Populations* (8.4)].

Because the biological activity of denosumab in animals is specific to nonhuman primates, evaluation of genetically engineered ("knockout") mice or use of other biological inhibitors of the RANK/RANKL pathway, namely OPG-Fc, provided additional information on the pharmacodynamic properties of denosumab. RANK/RANKL knockout mice exhibited absence of lymph node formation, as well as an absence of lactation due to inhibition of mammary gland maturation (lobulo-alveolar gland development during pregnancy). Neonatal RANK/RANKL knockout mice exhibited reduced bone growth and lack of tooth eruption. A corroborative study in 2-week-old rats given the RANKL inhibitor OPG-Fc also showed reduced bone growth, altered growth plates, and impaired tooth eruption. These changes were partially reversible in this model when dosing with the RANKL inhibitors was discontinued [see *Use in Specific Populations* (8.1, 8.4)].

14 CLINICAL STUDIES

14.1 Postmenopausal Women with Osteoporosis

The efficacy and safety of Prolia in the treatment of postmenopausal osteoporosis was demonstrated in a 3-year, randomized, double-blind, placebo-controlled trial. Enrolled women had a baseline BMD T-score between -2.5 and -4.0 at either the lumbar spine or total hip. Women with other diseases (such as rheumatoid arthritis, osteogenesis imperfecta, and Paget's disease) or on therapies that affect bone were excluded from this study. The 7808 enrolled women were aged 60 to 91 years with a mean age of 72 years. Overall, the mean baseline lumbar spine BMD T-score was -2.8 and 23% of women had a

vertebral fracture at baseline. Women were randomized to receive SC injections of either placebo (N = 3906) or Prolia 60 mg (N = 3902) once every 6 months. All women received at least 1000 mg calcium and 400 IU vitamin D supplementation daily.

The primary efficacy variable was the incidence of new morphometric (radiologically-diagnosed) vertebral fractures at 3 years. Vertebral fractures were diagnosed based on lateral spine radiographs (T4-L4) using a semiquantitative scoring method. Secondary efficacy variables included the incidence of hip fracture and nonvertebral fracture, assessed at 3 years.

Effect on Vertebral Fractures

Prolia significantly reduced the incidence of new morphometric vertebral fractures at 1, 2, and 3 years ($p < 0.0001$), as shown in Table 2. The incidence of new vertebral fractures at year 3 was 7.2% in the placebo-treated women compared to 2.3% for the Prolia-treated women. The absolute risk reduction was 4.8% and relative risk reduction was 68% for new morphometric vertebral fractures at year 3.

Table 2. The Effect of Prolia on the Incidence of New Vertebral Fractures

	Proportion of Women With Fracture (%) ⁺		Absolute Risk Reduction (%) [*] (95% CI)	Relative Risk Reduction (%) [*] (95% CI)
	Placebo N = 3691 (%)	Prolia N = 3702 (%)		
0-1 Year	2.2	0.9	1.4 (0.8, 1.9)	61 (42, 74)
0-2 Years	5.0	1.4	3.5 (2.7, 4.3)	71 (61, 79)
0-3 Years	7.2	2.3	4.8 (3.9, 5.8)	68 (59, 74)

* Absolute risk reduction and relative risk reduction based on Mantel-Haenszel method adjusting for age group variable.

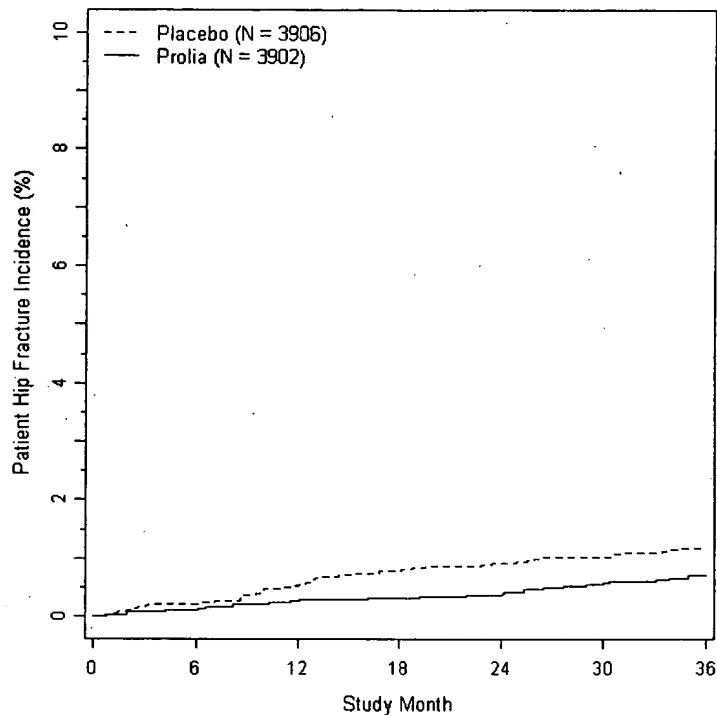
* Event rates based on crude rates in each interval.

Prolia was effective in reducing the risk for new morphometric vertebral fractures regardless of age, baseline rate of bone turnover, baseline BMD, baseline history of fracture, or prior use of a drug for osteoporosis.

Effect on Hip Fractures

The incidence of hip fracture was 1.2% for placebo-treated women compared to 0.7% for Prolia-treated women at year 3. The age-adjusted absolute risk reduction of hip fractures was 0.3% with a relative risk reduction of 40% at 3 years ($p = 0.04$) (Figure 1).

Figure 1. Cumulative Incidence of Hip Fractures Over 3 Years



N = number of subjects randomized

Effect on Nonvertebral Fractures

Treatment with Prolia resulted in a significant reduction in the incidence of nonvertebral fractures (Table 3).

Table 3. The Effect of Prolia on the Incidence of Nonvertebral Fractures at Year 3

	Proportion of Women With Fracture (%) ⁺		Absolute Risk Reduction (%) (95% CI)	Relative Risk Reduction (%) (95% CI)
	Placebo N = 3906 (%)	Prolia N = 3902 (%)		
Nonvertebral fracture [†]	8.0	6.5	1.5 (0.3, 2.7)	20 (5, 33) [*]

⁺ p-value = 0.01.

^{*} Event rates based on Kaplan-Meier estimates at 3 years.

[†] Excluding those of the vertebrae (cervical, thoracic, and lumbar), skull, facial, mandible, metacarpus, and finger and toe phalanges.

Effect on Bone Mineral Density (BMD)

Treatment with Prolia significantly increased BMD at all anatomic sites measured at 3 years. The treatment differences in BMD at 3 years were 8.8% at the lumbar spine, 6.4% at the total hip, and 5.2% at the femoral neck. Consistent effects on BMD were observed at the lumbar spine, regardless of baseline age, race, weight/body mass index (BMI), baseline BMD, and level of bone turnover.

After Prolia discontinuation, BMD returned to approximately baseline levels within 12 months.

Bone Histology and Histomorphometry

A total of 115 transiliac crest bone biopsy specimens were obtained from 92 postmenopausal women with osteoporosis at either month 24 and/or month 36 (53 specimens in Prolia group, 62 specimens in placebo group). Of the biopsies obtained, 115 (100%) were adequate for qualitative histology and 7 (6%) were adequate for full quantitative histomorphometry assessment.

Qualitative histology assessments showed normal architecture and quality with no evidence of mineralization defects, woven bone, or marrow fibrosis in patients treated with Prolia.

The presence of double tetracycline labeling in a biopsy specimen provides an indication of active bone remodeling, while the absence of tetracycline label suggests suppressed bone formation. In subjects treated with Prolia, 35% had no tetracycline label present at the month 24 biopsy and 38% had no tetracycline label present at the month 36 biopsy, while 100% of placebo-treated patients had double label present at both time points. When compared to placebo, treatment with Prolia resulted in virtually absent activation frequency and markedly reduced bone formation rates. However, the long-term consequences of this degree of suppression of bone remodeling are unknown.

16 HOW SUPPLIED/STORAGE AND HANDLING

Prolia is supplied in a single-use prefilled syringe with a safety guard or in a single-use vial. The grey needle cap on the single-use prefilled syringe contains dry natural rubber (a derivative of latex).

60 mg/1 mL in a single-use prefilled syringe	1 per carton	NDC 55513-710-01
60 mg/1 mL in a single-use vial	1 per carton	NDC 55513-720-01

Store Prolia in a refrigerator at 2°C to 8°C (36°F to 46°F) in the original carton. Do not freeze. Prior to administration, Prolia may be allowed to reach room temperature (up to 25°C/77°F) in the original container. Once removed from the refrigerator, Prolia must not be exposed to temperatures above 25°C/77°F and must be used within 14 days. If not used within the 14 days, Prolia should be discarded. Do not use Prolia after the expiry date printed on the label.

Protect Prolia from direct light and heat.

Avoid vigorous shaking of Prolia.

17 PATIENT COUNSELING INFORMATION

See Medication Guide.

17.1 Hypocalcemia

Adequately supplement patients with calcium and vitamin D and instruct them on the importance of maintaining serum calcium levels while receiving Prolia [see *Warnings and Precautions (5.1)* and *Use in Specific Populations (8.6)*]. Advise patients to seek prompt medical attention if they develop signs or symptoms of hypocalcemia.

17.2 Serious Infections

Advise patients to seek prompt medical attention if they develop signs or symptoms of infections, including cellulitis [see *Warnings and Precautions* (5.2)].

17.3 Dermatologic Reactions

Advise patients to seek prompt medical attention if they develop signs or symptoms of dermatological reactions (dermatitis, rashes, and eczema) [see *Warnings and Precautions* (5.3)].

17.4 Osteonecrosis of the Jaw

Advise patients to maintain good oral hygiene during treatment with Prolia and to inform their dentist prior to dental procedures that they are receiving Prolia. Patients should inform their physician or dentist if they experience persistent pain and/or slow healing of the mouth or jaw after dental surgery [see *Warnings and Precautions* (5.4)].

17.5 Schedule of Administration

If a dose of Prolia is missed, administer the injection as soon as convenient. Thereafter, schedule injections every 6 months from the date of the last injection.



Manufactured by:

Amgen Manufacturing Limited, a subsidiary of Amgen Inc.
One Amgen Center Drive
Thousand Oaks, California 91320-1799

This product, its production, and/or its use may be covered by one or more US Patents, including US Patent Nos. 6,740,522; 7,097,834; 7,364,736; and 7,411,050, as well as other patents or patents pending.

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ATTACHMENT E

In re U.S. Patent No. 6,740,522

Issued: May 25, 2004

To: Dirk M. Anderson

Assignee: Immunex Corporation

**For: ANTIBODIES AGAINST LIGAND FOR RECEPTOR
ACTIVATOR OF NF-KB**

Application for Patent Term Extension

Customer No. 22852

DESCRIPTION OF REGULATORY ACTIVITIES

<u>Date</u>	<u>Description</u>
22-May-2001	Initial IND Application: POSTMENOPAUSAL OSTEOPOROSIS
20-Jun-2001	Amgen teleconference
26-Jul-2001	Amgen phone contact
10-Sep-2001	Amgen fax preparation for teleconference 24 September 2001
24-Sep-2001	Amgen teleconference
8-Nov-2001	Review commitment, nonclinical study report
8-Nov-2001	Information Amendment
27-Nov-2001	Information Amendment
18-Dec-2001	Amgen teleconference
20-Dec-2001	Information Amendment
9-Jan-2002	Amgen teleconference phase 2 studies
15-Jan-2002	Response To Questions Proposal for Phase II Study
16-Jan-2002	Amgen teleconference
6-Feb-2002	Protocol Amendment
14-Feb-2002	Response To Questions
14-Feb-2002	Manufacturing change
5-Mar-2002	Protocol Amendment 2
5-Mar-2002	Information Amendment
11-Mar-2002	Response To Questions
2-Apr-2002	Protocol Amendment 1
5-Apr-2002	Response To Questions IND Review Jul 17, 2001
23-Sep-2002	Protocol Amendment 2
28-Oct-2002	Protocol Amendment 3
6-Nov-2002	Manufacturing change
27-Dec-2002	Information Amendment
6-Jan-2003	2002 Annual Report
24-Jan-2003	Protocol Amendment 4
3-Feb-2003	Safety Report
23-Jun-2003	Protocol Amendment
30-Jul-2003	Protocol Amendment 1
25-Aug-2003	2003 Annual Report
25-Aug-2003	Safety Report
26-Aug-2003	Safety Report
26-Aug-2003	Safety Report
5-Sep-2003	Safety Report
18-Sep-2003	2003 Annual Report Addendum
25-Sep-2003	Safety Report
3-Nov-2003	Protocol Amendment 1
11-Dec-2003	Protocol Amendment 5
19-Feb-2004	Meeting Request - End of Phase 2 Type B Meeting
20-Feb-2004	Meeting Request

12-Mar-2004	Briefing Document
19-Mar-2004	Briefing materials
27-Apr-2004	Manufacturing change
27-Apr-2004	Protocol Amendment 3
30-Apr-2004	Meeting Minutes
11-May-2004	Safety Report
20-May-2004	Agency meeting summary letter
2-Jun-2004	Protocol Amendment
4-Jun-2004	Response To Questions of 20 May 2004
14-Jun-2004	Amgen teleconference
15-Jun-2004	Safety Report
17-Jun-2004	Protocol Amendment 6
17-Jun-2004	Amgen teleconference
21-Jun-2004	Response To Questions
23-Jun-2004	Meeting Minutes
12-Jul-2004	Safety Report
13-Jul-2004	Amendment to Type C Meeting - CP2 Product characterization data
20-Jul-2004	Protocol Amendment
21-Jul-2004	Information Amendment 4
26-Jul-2004	Safety Report
3-Aug-2004	Information Amendment
5-Aug-2004	Protocol Amendment 2
5-Aug-2004	Safety Report
23-Aug-2004	Safety Report
23-Aug-2004	Amgen teleconference
21-Sep-2004	Amgen phone contact
28-Sep-2004	Information Amendment
12-Oct-2004	Meeting Minutes from 29 September 2004
29-Oct-2004	Meeting Minutes from 09/21/2004 Telecon
3-Nov-2004	Annual Report 2004
21-Dec-2004	Information Amendment
28-Jan-2005	Amgen phone contact
31-Jan-2005	Response To Questions
31-Jan-2005	Amgen phone contact
8-Feb-2005	Amgen phone contact
14-Feb-2005	Amgen phone contact
9-Mar-2005	Protocol Amendment 1
14-Mar-2005	Type C Meeting Request
21-Mar-2005	Safety Report
23-Mar-2005	Safety Report
28-Mar-2005	Safety Report
8-Apr-2005	Safety Report
14-Apr-2005	Manufacturing change
15-Apr-2005	Safety Report
25-Apr-2005	Amgen phone contact
28-Apr-2005	Safety Report
28-Apr-2005	Safety Report

4-May-2005	Safety Report
5-May-2005	Amgen phone contact
6-May-2005	Response to Questions of 21 APR 2005
12-May-2005	Briefing Document Phase III Study Design
12-May-2005	Safety Report
19-May-2005	Safety Report
24-May-2005	Agency Clinical & Statistical comments
7-Jun-2005	Safety Report
9-Jun-2005	Response To Questions of 21 APR
15-Jun-2005	Safety Report
18-Jun-2005	Type C Clinical Meeting 18 June 2005
28-Jun-2005	Protocol Amendment 1
30-Jun-2005	Information Amendment
8-Jul-2005	Safety Report
22-Jul-2005	Protocol Amendment 2
18-Aug-2005	Information Amendment 5
2-Sep-2005	Information Amendment
27-Sep-2005	Safety Report
5-Oct-2005	Response To Questions 24 May 2005
12-Oct-2005	Safety Report
17-Oct-2005	Safety Report
18-Oct-2005	Safety Report
19-Oct-2005	Information Amendment
19-Oct-2005	Safety Report
20-Oct-2005	Safety Report
25-Oct-2005	Information Amendment
26-Oct-2005	Safety Report
3-Nov-2005	Safety Report
11-Nov-2005	Safety Report
15-Nov-2005	Safety Report
16-Nov-2005	Safety Report
17-Nov-2005	Safety Report
18-Nov-2005	Safety Report
21-Nov-2005	Safety Report
30-Nov-2005	Safety Report
6-Dec-2005	Safety Report
9-Dec-2005	Safety Report
12-Dec-2005	Annual Report 24 June 2004 - 23 June 2005
15-Dec-2005	Protocol Amendment
20-Dec-2005	Switch to eCTD Format
23-Dec-2005	Safety Report
23-Dec-2005	Safety Report
4-Jan-2006	Safety Report
6-Jan-2006	Protocol Amendment 1
17-Jan-2006	Safety Report
20-Jan-2006	Safety Report
25-Jan-2006	Protocol Amendment

30-Jan-2006	Type C Meeting Request
31-Jan-2006	Safety Report
2-Feb-2006	Safety Report
3-Feb-2006	Meeting Request
3-Feb-2006	Safety Report
9-Feb-2006	Safety Report
14-Feb-2006	Safety Report
15-Feb-2006	Protocol Amendment
17-Feb-2006	Safety Report
22-Feb-2006	Safety Report
23-Feb-2006	Safety Report
24-Feb-2006	Safety Report
28-Feb-2006	Protocol Amendment
3-Mar-2006	Safety Report
6-Mar-2006	Safety Report
13-Mar-2006	Information Amendment
15-Mar-2006	Safety Report
17-Mar-2006	Safety Report
21-Mar-2006	Information Amendment
24-Mar-2006	Briefing Document for Meeting scheduled 24 April 2006
24-Mar-2006	Safety Report
29-Mar-2006	Safety Report
31-Mar-2006	Safety Report
3-Apr-2006	Withdrawal of Type C CMC Meeting Request
6-Apr-2006	Re-submission of non-clinical documents
7-Apr-2006	Safety Report
12-Apr-2006	Safety Report
14-Apr-2006	Information Amendment
18-Apr-2006	Safety Report
20-Apr-2006	Response To Questions
21-Apr-2006	Information Amendment
28-Apr-2006	Protocol Amendment
2-May-2006	Safety Report
5-May-2006	Information Amendment
8-May-2006	Safety Report
12-May-2006	Protocol Amendment
15-May-2006	Safety Report
18-May-2006	Protocol Amendment
22-May-2006	Safety Report
24-May-2006	Response To Questions
25-May-2006	Tox reports Studies
25-May-2006	Safety Report
2-Jun-2006	Safety Report
8-Jun-2006	Information Amendment
8-Jun-2006	Safety Report
21-Jun-2006	Annual Report 2006 06-24-2005 thru 03-24-2006

29-Jun-2006	Safety Report
11-Jul-2006	Safety Report
17-Jul-2006	Safety Report
19-Jul-2006	Safety Report
27-Jul-2006	Safety Reports
28-Jul-2006	Protocol Amendment
28-Jul-2006	General Correspondence
9-Aug-2006	Protocol Amendment
10-Aug-2006	Safety Report
11-Aug-2006	General Correspondence
23-Aug-2006	Safety Report
25-Aug-2006	Amgen fax Type C Meeting Request Draft
31-Aug-2006	Safety Report
1-Sep-2006	Protocol Amendment
8-Sep-2006	Safety Report
12-Sep-2006	Safety Report
15-Sep-2006	General Correspondence - Type C Meeting Request
21-Sep-2006	Information Amendment
5-Oct-2006	Safety Report
11-Oct-2006	Safety Report
11-Oct-2006	Protocol Amendment
8-Nov-2006	Briefing Document for Type C Meeting
10-Nov-2006	Protocol Amendment
13-Nov-2006	Protocol Amendment
17-Nov-2006	Safety Report
22-Nov-2006	Safety Report
28-Nov-2006	Amgen email premeeting comments
1-Dec-2006	Amgen email request for information
7-Dec-2006	Safety Report
7-Dec-2006	Safety Report
8-Dec-2006	Amgen email
8-Dec-2006	Type C CMC Meeting
11-Dec-2006	Safety Report
15-Dec-2006	General Correspondence
20-Dec-2006	Response to Questions pre-clinical study reports - 16 November 2006
21-Dec-2006	Amgen email regarding 08 December 2006 Meeting Minutes
22-Dec-2006	Safety Report
3-Jan-2007	Safety Report
4-Jan-2007	Safety Reports
8-Jan-2007	Amgen email regarding Informed Consent submitted
9-Jan-2007	General Correspondence Meeting Minutes 8 Dec. 2006
16-Jan-2007	Safety Report
18-Jan-2007	Safety Report
23-Jan-2007	Safety Report
30-Jan-2007	Amgen email
31-Jan-2007	Amgen phone
31-Jan-2007	Safety Report

31-Jan-2007	General Correspondence
13-Feb-2007	Safety Report
15-Feb-2007	Safety Report
21-Feb-2007	Protocol Amendment
27-Feb-2007	Safety Report
1-Mar-2007	Safety Report
2-Mar-2007	Information Amendment Plan
16-Mar-2007	Amgen email
19-Mar-2007	Information Amendment Plan
20-Mar-2007	Safety Report
22-Mar-2007	General Correspondence
22-Mar-2007	Safety Report
22-Mar-2007	Amgen email and phone conversation
30-Mar-2007	Safety Report
3-Apr-2007	Protocol Amendment Data
4-Apr-2007	Safety Report
3-Apr-2007	Amgen contact
4-Apr-2007	General Correspondence
11-Apr-2007	Safety Report
17-Apr-2007	Safety Report
18-Apr-2007	Protocol Amendment
18-Apr-2007	Safety Report
20-Apr-2007	General Correspondence
24-Apr-2007	Safety Report
25-Apr-2007	Safety Report
30-Apr-2007	Safety Report
1-May-2007	Safety Report
4-May-2007	Amgen Record of Contact Preliminary Questions regarding 6 June 2007 Meeting
4-May-2007	Safety Report
8-May-2007	Safety Report
8-May-2007	Briefing Document Type C Meeting 6 June 2007
10-May-2007	Safety Report
1-Jun-1007	Information Amendment
22-May-2007	Safety Report
23-May-2007	Safety Report
24-May-2007	Information Amendment
29-May-2007	Information Amendment
29-May-2007	Safety Report
6-Jun-2007	Type C Meeting Teleconference
6-Jun-2007	Safety Report
6-Jun-2007	Safety Report
14-Jun-2007	Response To Questions from Agency
13-Jun-2007	Safety Report
20-Jun-2007	Annual Report 2006 03-25-2006 thru 03-23-2007 & stability data
19-Jun-2007	Meeting Minutes from 06 June 2007 Type C Meeting
20-Jun-2007	General Correspondence

21-Jun-2007	Protocol Amendment
21-Jun-2007	Safety Report
22-Jun-2007	Protocol Amendment
25-Jun-2007	Safety Report
3-Jul-2007	Safety Report
10-Jul-2007	Safety Report
11-Jul-2007	Safety Report
12-Jul-2007	Protocol Amendment
12-Jul-2007	Safety Report
13-Jul-2007	Safety Report
16-Jul-2007	Safety Report
24-Jul-2007	Information Amendment
23-Jul-2007	Safety Report
24-Jul-2007	Safety Report
27-Jul-2007	Protocol Amendment
26-Jul-2007	Safety Report
3-Aug-2007	Safety Report
7-Aug-2007	Safety Report
17-Aug-2007	Protocol Amendment
21-Aug-2007	Safety Report
28-Aug-2007	Information Amendment
28-Aug-2007	General Correspondence
24-Aug-2007	Safety Report
28-Aug-2007	Safety Report
30-Aug-2007	Safety Report
5-Sep-2007	Information Amendment
6-Sep-2007	Safety Report
12-Sep-2007	Safety Report
21-Sep-2007	Safety Report
13-Sep-2007	Safety Report
14-Sep-2007	Response to Questions
14-Sep-2007	Safety Report
18-Sep-2007	Safety Report
20-Sep-2007	Safety Report
25-Sep-2007	Safety Report
28-Sep-2007	Safety Report
1-Oct-2007	Safety Report
2-Oct-2007	Protocol Amendment
2-Oct-2007	Safety Report
3-Oct-2007	Safety Report
4-Oct-2007	Safety Report
10-Oct-2007	Safety Report
11-Oct-2007	Safety Report
12-Oct-2007	Safety Report
15-Oct-2007	General Correspondence
15-Oct-2007	Safety Report
16-Oct-2007	Safety Report

17-Oct-2007	Safety Report
18-Oct-2007	Safety Report
18-Oct-2007	Safety Report
22-Oct-2007	Safety Report
26-Oct-2007	Safety Report
23-Oct-2007	Safety Report
24-Oct-2007	Safety Report
1-Nov-2007	Safety Report
5-Nov-2007	Information Amendment
7-Nov-2007	Safety Report
8-Nov-2007	Safety Report
9-Nov-2007	Protocol Amendment
13-Nov-2007	Safety Report
19-Nov-2007	General Correspondence - Type C Meeting Request
14-Nov-2007	Safety Report
19-Nov-2007	Safety Report
20-Nov-2007	Safety Report
27-Nov-2007	Safety Report
28-Nov-2007	Safety Report
29-Nov-2007	Safety Report
30-Nov-2007	Safety Report
7-Dec-2007	Information Amendment
7-Dec-2007	Protocol Amendment
5-Dec-2007	Safety Report
6-Dec-2007	Safety Report
19-Dec-2007	General Correspondence Briefing Document
11-Dec-2007	Safety Report
13-Dec-2007	Protocol Amendment
13-Dec-2007	Safety Report
18-Dec-2007	Safety Report
19-Dec-2007	Safety Report
20-Dec-2007	Safety Report
21-Dec-2007	Safety Report
2-Jan-2008	Safety Report
3-Jan-2008	Safety Report
8-Jan-2008	Information Amendment
8-Jan-2008	Information Amendment
8-Jan-2008	Safety Report
11-Jan-2008	Information Amendment
10-Jan-2008	Safety Report
15-Jan-2008	Safety Report
18-Jan-2008	Safety Report
25-Jan-2008	General Correspondence
23-Jan-2008	Safety Report
24-Jan-2008	Safety Report
25-Jan-2008	Safety Report
29-Jan-2008	Safety Report

30-Jan-2008	Safety Report
31-Jan-2008	Safety Report
4-Feb-2008	Safety Report
5-Feb-2008	Type C Meeting Teleconference
5-Feb-2008	Safety Report
12-Feb-2008	Protocol Amendment
6-Feb-2008	Safety Report
7-Feb-2008	Safety Report
11-Feb-2008	Safety Report
13-Feb-2008	General Correspondence Meeting Minutes 5 February 2008
12-Feb-2008	Safety Report
13-Feb-2008	Safety Report
14-Feb-2008	Safety Report
18-Feb-2008	Safety Report
20-Feb-2008	Safety Report
26-Feb-2008	Protocol Amendment
26-Feb-2008	Safety Report
27-Feb-2008	Safety Report
28-Feb-2008	Safety Report
29-Feb-2008	Safety Report
12-Mar-2008	Information Amendment
11-Mar-2008	Safety Report
13-Mar-2008	Safety Report
17-Mar-2008	Safety Report
19-Mar-2008	Information Amendment
21-Mar-2008	Safety Report
25-Mar-2008	Safety Report
25-Mar-2008	Information Amendment
26-Mar-2008	Safety Report
27-Mar-2008	Safety Report
31-Mar-2008	Safety Report
2-Apr-2008	Safety Report
22-Apr-2008	General Correspondence Type B CMC Meeting Request
9-Apr-2008	Safety Report
11-Apr-2008	Protocol Amendment
11-Apr-2008	General Correspondence
17-Apr-2008	Information Amendment
16-Apr-2008	Safety Report
17-Apr-2008	Safety Report
24-Apr-2008	Safety Report
1-May-2008	Protocol Amendment
29-Apr-2008	Safety Report
1-May-2008	Safety Report
5-May-2008	General Correspondence-Cross Reference to IND 9838 Safety Response to Questions
7-May-2008	Safety Report
9-May-2008	Information Amendment
13-May-2008	Information Amendment

16-May-2008	General Correspondence
23-May-2008	Safety Report
27-May-2008	Safety Report
29-May-2008	Information Amendment
29-May-2008	Information Amendment
30-May-2008	Safety Report
3-Jun-2008	General Correspondence
4-Jun-2008	Safety Report
13-Jun-2008	General Correspondence Pre-BLA Meeting Request
11-Jun-2008	Safety Report
17-Jun-2008	Safety Report
19-Jun-2008	Safety Report
20-Jun-2008	Annual Report 2008 03-24-2007 thru 03-24-2008
19-Jun-2008	Safety Report
27-Jun-2008	General Correspondence
9-Jul-2008	Safety Report
16-Jul-2008	Information Amendment
16-Jul-2008	General Correspondence
18-Jul-2008	General Correspondence
21-Jul-2008	Safety Report
22-Jul-2008	Information Amendment
30-Jul-2008	General Correspondence
25-Jul-2008	General Correspondence
29-Jul-2008	Safety Report
29-Jul-2008	Type B Pre BLA CMC Meeting Follow-up Teleconference
1-Aug-2008	General Correspondence
4-Aug-2008	Information Amendment
3-Aug-2008	Safety Report
25-Aug-2008	Information Amendment
26-Aug-2008	Safety Report
3-Sep-2008	Protocol Amendment
3-Sep-2008	Safety Report
4-Sep-2008	General Correspondence
5-Sep-2008	Safety Report
11-Sep-2008	General Correspondence Type B Pre-BLA Briefing Document
15-Sep-2008	Safety Report
19-Sep-2008	Safety Report
22-Sep-2008	Protocol Amendment
22-Sep-2008	Safety Report
29-Sep-2008	Information Amendment
16-Oct-2008	Safety Report
21-Oct-2008	Type B Pre-BLA Meeting
24-Oct-2008	General Correspondence Type B Pre-BLA Meeting Minutes 21 October 2008
27-Oct-2008	General Correspondence
30-Oct-2008	Information Amendment
6-Nov-2008	Safety Report
18-Nov-2008	General Correspondence

19-Nov-2008	General Correspondence
3-Dec-2008	Safety Report
11-Dec-2008	General Correspondence
12-Dec-2008	Information Amendment
19-Dec-2008	BLA 125320/0/0
16-Dec-2008	Safety Report
30-Dec-2008	Safety Report
7-Jan-2009	General Correspondence
15-Jan-2009	General Correspondence
16-Jan-2009	Safety Report
22-Jan-2009	General Correspondence
29-Jan-2009	General Correspondence
5-Feb-2009	Safety Report
9-Feb-2009	General Correspondence
20-Feb-2009	General Correspondence
23-Feb-2009	Information: Clinical safety report notifications
26-Feb-2009	Information Amendment
3-Mar-2009	General Correspondence
3-Mar-2009	Safety Report
10-Mar-2009	Information Amendment
18-Mar-2009	Protocol Amendment
20-Mar-2009	Protocol Amendment
3-Apr-2009	Type C Meeting Briefing Document
24-Mar-2009	Safety Report
6-Apr-2009	Protocol Amendment
2-Apr-2009	Safety Report
8-Apr-2009	Safety Report
14-Apr-2009	Safety Report
16-Apr-2009	Safety Report
20-Apr-2009	Safety Report
24-Apr-2009	Safety Report
28-Apr-2009	Protocol Amendment
5-May-2009	Safety Report
13-May-2009	Quarterly Safety Update Report
19-May-2009	Information Amendment
19-May-2009	Safety Report
29-May-2009	General Correspondence
3-Jun-2009	Type C Meeting -MOP
3-Jun-2009	General Correspondence
2-Jun-2009	Safety Report
11-Jun-2009	Safety Report
22-Jun-2009	Annual Report 2009 03-24-2008 thru 03-24-2009
18-Jun-2009	Safety Report
22-Jun-2009	General Correspondence
23-Jun-2009	Safety Report
7-Jul-2009	General Correspondence
29-Jun-2009	Safety Report

7-Jul-2009	Safety Report
13-Jul-2009	General Correspondence
13-Jul-2009	Safety Report
22-Jul-2009	Protocol Amendment
30-Jul-2009	Information Amendment
4-Aug-2009	General Correspondence
23-Jul-2009	Safety Report
23-Jul-2009	General Correspondence
4-Aug-2009	Safety Report
2-Sep-2009	Safety Report
7-Aug-2009	Safety Report
12-Aug-2009	Quarterly Safety Update Report
20-Aug-2009	Safety Report
25-Aug-2009	Safety Report
1-Sep-2009	Safety Report
4-Sep-2009	Safety Report
21-Sep-2009	Information Amendment
10-Sep-2009	Safety Report
16-Sep-2009	Safety Report
17-Sep-2009	Safety Report
18-Sep-2009	General Correspondence
22-Sep-2009	Safety Report
25-Sep-2009	Safety Report
29-Sep-2009	Safety Report
1-Oct-2009	Safety Report
8-Oct-2009	Safety Report
13-Oct-2009	Safety Report
14-Oct-2009	Safety Report
20-Oct-2009	Safety Report
21-Oct-2009	Safety Report
22-Oct-2009	Safety Report
29-Oct-2009	Safety Report
4-Nov-2009	Quarterly Safety Update Report
3-Nov-2009	Safety Report
5-Nov-2009	Safety Report
6-Nov-2009	Safety Report
13-Nov-2009	Safety Report
18-Nov-2009	Safety Report
19-Nov-2009	Safety Report
24-Nov-2009	Information Amendment
23-Nov-2009	Safety Report
25-Nov-2009	Safety Report
4-Dec-2009	Safety Report
7-Dec-2009	Safety Report
16-Dec-2009	Safety Report
18-Dec-2009	Safety Report
22-Dec-2009	Safety Report

23-Dec-2009	Safety Report
5-Jan-2010	Safety Report
11-Jan-2010	Safety Report
15-Jan-2010	Safety Report
20-Jan-2010	Safety Report
22-Jan-2010	Safety Report
26-Jan-2010	Safety Report
2-Feb-2010	Safety Report
11-Feb-2010	Quarterly Safety Update Report
9-Feb-2010	General Correspondence
8-Feb-2010	Safety Report
9-Feb-2010	Safety Report
11-Feb-2010	Information Amendment
15-Feb-2010	Safety Report
16-Feb-2010	General Correspondence
16-Feb-2010	Safety Report
19-Feb-2010	Safety Report
25-Feb-2010	Safety Report
2-Mar-2010	General Correspondence
5-Mar-2010	Response to Questions: Statistical Analysis Plan
10-Mar-2010	Safety Report
24-Mar-2010	Safety Report
1-Apr-2010	Safety Report
5-Apr-2010	Information Amendment CMC
6-Apr-2010	Safety Report
8-Apr-2010	Safety Report
12-Apr-2010	Safety Report
14-Apr-2010	Safety Report
20-Apr-2010	Safety Report
29-Apr-2010	General Correspondence
27-Apr-2010	Safety Report
5-May-2010	Quarterly Safety Update Report
30-Apr-2010	Information Amendment
4-May-2010	Safety Report
21-May-2010	Safety Report
28-May-2010	Safety Report
1-Jun-2010	BLA Approved